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Exercise is beneficial to cognitive performance and to executive function (EF) in particular, however the underlying mechanisms as to how exercise yields these benefits are not clear. The overall purpose of this study was to identify the extent to which a single bout of exercise affects EF in older adults and to gain an understanding of the role of biological factors such as vagal tone, brain-derived neurotrophic factor (BDNF), and the hemodynamic response in neural tissues (i.e. neural activation patterns) in this relationship. Vagal tone, a measure of parasympathetic nervous system activity, shares an intimate relationship with EF in adults; higher resting vagal tone is associated with better EF and increased vagal tone following exercise training is associated with improved EF. Interestingly, BDNF and neural activation patterns respond to acute exercise and are related to both vagal tone and EF in older adults. As such we investigated BDNF and neural activation as mechanisms in the relationship of acute exercise and EF in older adults with vagal tone as a potential moderator. Participants were sixteen healthy, older adults (M=72.3 years) from the community. Day one testing consisted of an assessment of resting heart rate, EF testing (inhibitory control, set shifting, and working memory), a blood draw, and a submaximal exercise test. Days two and three were counterbalanced for condition (exercise or rest) and included a pre-condition blood draw, exercise or rest, a post-condition blood draw, and post-condition EF testing (same as baseline) during a functional

magnetic resonance imaging (fMRI) scan. The exercise condition included cycling at 55-65% Heart Rate Reserve for 30 minutes and the rest condition consisted of sitting on the bike for 30 minutes at rest. Paired samples t-tests assessed the effects of condition on EF, BDNF, and neural activation. Linear regressions were used to assess the relationships between change in BDNF and change in neural activation with change in EF. A median split was performed on vagal tone (root mean square of the successive differences; RMSSD) and independent samples t-tests assessed the difference between high and low vagal tone groups on change in BDNF, change in neural activation, and change in EF. There was no overall effect of condition on change in EF or change in BDNF, however change in BDNF following exercise, relative to following rest, predicted change in set shifting. There were significant differences in neural activation following exercise, compared to rest, and change in neural activation was associated with EF. Interestingly, those with higher vagal tone had greater benefits from exercise as compared to those with lower vagal tone as assessed using measures of EF, BDNF, and neural activation. These findings provide initial evidence of mechanisms involved in the relationship of acute exercise and EF in older adults. Of importance, we identified vagal tone as a moderator that helps to account for some individual variability in the response to exercise. Larger studies are needed to test these preliminary relationships in a moderated-mediation model.

EXERCISE AND EXECUTIVE FUNCTION IN OLDER ADULTS:

EXPLORING THE MECHANISMS

by

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CHAPTER I

INTRODUCTION

Statement of the Problem

By 2030, one in every five American adults will be over the age of 65 and at an increased risk for age-related cognitive decline (US Census Bureau, 2010). Though many cognitive abilities decline throughout middle and older adulthood, declines in executive function (i.e. inhibitory control, set shifting, and working memory; Miyake et al., 2000) precede declines in memory (Carlson et al., 2008; Harrington et al., 2013) and global cognitive decline (Clark et al., 2012). Further, declines in executive function impact older adults' quality of life (Forte, Boreham, de Vito, & Pesce, 2015) and are associated with declines in functional ability (Johnson, Lui, & Yaffe, 2007; Tomaszewski Farias et al., 2009) and all-cause mortality (Johnson et al., 2007; Peng et al., 2016). Although declines in executive function are a common course of aging, meta-analytic reviews show that acute (i.e., a single bout) and chronic exercise have a positive effect on executive function in older adults (Colcombe & Kramer, 2003; Ludyga, Gerber, Brand, Holsboer-Trachsler, & Pühse, 2016). Still, the underlying origin of the acute effects, which may extend to cumulative effects on executive function remains unclear. Given the importance of executive function for the well-being of the

aging population and the potential for exercise to have a positive impact, it is of great importance to understand the mechanism(s) of the effect of acute exercise on executive function in older adults in order to maximize the potential benefits.

Acute exercise induces a transient physiological response, whereas chronic exercise induces adaptations to exercise that affect the physiological systems at rest (Etnier et al., 1997). Some suggest that these results are complimentary, such that the effect of chronic exercise is merely an accumulation of repeated responses to acute bouts of exercise. Acute exercise benefits older adults' executive function (Ludyga et al., 2016), but understanding the mechanism(s) of how acute exercise affects executive function is an important first step towards creating exercise prescriptions designed to improve executive function and/or overall cognition in older adults. Two primary mechanisms of interest here are brain-derived neurotrophic factor (BDNF) and the hemodynamic response in neural tissues (herein neural activation).

BDNF plays a critical role in neuroprotection, neurogenesis, and synaptic plasticity and is decreased throughout older adulthood (Marosi & Mattson, 2014). BDNF is considered a bioenergetic protein because BDNF expression increases in response to energetic challenges, such as caloric restriction and exercise (Dinoff, Herrmann, Swardfager, & Lanctôt, 2017; Mattson, Maudsley, & Martin, 2004). In rodents, BDNF expression increases in response to exercise within brain regions associated with memory and EF, such as the hippocampus (Berchtold, Kesslak, Pike, Adlard, & Cotman, 2001) and prefrontal cortex (Geng,

Yu, & Wang, 2013), which provides basic support for the role of BDNF as a potential mediator in exercise and cognition.

Although BDNF expression in the brain is not easily measured in humans, centrally-derived BDNF is associated with peripheral BDNF at rest (Klein et al., 2011; Sartorius et al., 2009) and is a major contributor to increases in peripheral BDNF following acute exercise (Rasmussen et al., 2009). Further, increased BDNF following acute exercise is associated with improved executive function in young (Hwang et al., 2016) and older (Håkansson et al., 2017) adults; however, with young adults there is also evidence to the contrary (Ferris, Williams, & Shen, 2007; Tsai et al., 2014). Given that acute exercise transiently increases BDNF and there is support for the association between increased BDNF and improved executive function, the literature warrants further investigation into BDNF as a key mediator in the exercise and executive function relationship in older adults.

In addition to BDNF, a second mechanism to consider in the relationship of exercise and executive function is neural activation. The network of brain regions associated with performance of executive function includes the prefrontal cortex, frontopolar cortex, orbitofrontal cortex, and the anterior cingulate cortex (herein cognitive control network; Niendam et al., 2012). Throughout aging, neural activation, structural integrity, and connectivity of this network is altered, (Fjell et al., 2017; Li et al., 2015) and changes in connectivity in the cognitive control network account for about 83% of the age-related declines in executive function (Fjell et al., 2017). Though there is much evidence for the chronic effects

of exercise on brain structure (Colcombe et al., 2004; Colcombe et al., 2006; Erickson et al., 2011; Voss et al., 2013) and function (Colcombe et al., 2004; Voss et al., 2010) in older adults, the literature is very limited with respect to the acute effects.

To date, only three studies have utilized fMRI when investigating the effects of acute exercise on executive function and these have been conducted in children (Chen, Zhu, Yan, & Yin, 2016), adolescents with bipolar disorder (Metcalfe, MacIntosh, Scavone, Ou, & Goldstein, 2015), and young adults (Li et al., 2014). In response to acute exercise, children showed increased activation in bilateral superior parietal lobes, left hippocampus, and bilateral cerebellum, as well as improved working memory performance (Chen et al., 2016). Following acute exercise, bipolar adolescents, who normally exhibit altered neural activity in the accumbens compared to healthy controls, displayed similar activation to the controls (Metcalfe et al., 2015), suggesting a potential benefit of exercise on executive function through normalization of activation. Li et al. (2014) found that young adult females increased activation in frontal areas and decreased activation in the anterior cingulate cortex in response to acute exercise. Taken together, the available evidence is in support of acute exercise affecting neural activation and executive function in children, bipolar adolescents, and young adults. There is a need to pursue this mechanism with older adults as aging is associated with an increased vulnerability to changes in the cognitive control

network associated with declines in executive function (Fjell et al., 2017; Li et al., 2015).

Although there is evidence to suggest that BDNF and neural activation are important in the relation between exercise and executive function, it is also important to recognize that these general effects are subject to individual variability. Older adults have high variability in brain structure, brain function, and cognitive performance; suggested to reflect a variability in biological, psychological, and environmental factors as well as health-related behaviors (Glisky, 2007). The cognitive reserve theory suggests that cognitive reserves include both neural reserves (i.e. cognitive processing) and neural compensation (i.e. alterations to processing to cope with insults or pathology) and those with greater cognitive reserves (i.e. higher processing capacity and/or better ability to compensate) are more resilient to age and pathology-related declines (Stern, 2009). Importantly, those with reduced reserves have a greater window for improvement and may have a greater response to a stimulus (e.g. exercise) than someone with higher reserves; however, those with greater reserves would still be expected to outperform those with lesser reserves (Stern, 2009). When measuring the average response to acute exercise, BDNF increases, neural activation changes, and executive function is improved; however, there is individual variability in responses and as such there has been a recent interest in identifying exercise responders and non-responders (Bonafiglia et al., 2016; Yamazaki et al., 2017). Understanding the variability in responses is needed to

fully understand the mechanisms; the cognitive reserve theory provides a framework for inter-individual variability in response to exercise.

To date, in the only acute exercise study with healthy older adults in which changes in BDNF and executive function were assessed, Håkansson et al., (2017) found that the change in BDNF in response to exercise was associated with executive function for older adults, however the effect was specific to those with lower resting BDNF. This may suggest that those who start off in a poorer state (i.e. less reserves) may be dependent on the change in BDNF to achieve benefits in executive function. Given this evidence, we hypothesized that those with lower reserves, compared to those with higher reserves, would have a greater response in BDNF to exercise and the response would be associated with change in executive function.

The cognitive reserve theory also provides a framework for neural activation as an important factor in the relationship of exercise and executive function for older adults. Age-related changes to the functional integrity of neural networks affects neural reserves (Andrews-Hanna et al., 2007), consistent with the cognitive reserve theory, which may influence the necessity and/or effectiveness of neural compensation. Compensatory mechanisms include increasing activation or changing resource allocation (i.e. altered activation patterns) to tasks during performance to maintain or improve performance. However, if activation is reduced and performance is maintained or improved, it

would be indicative of neural efficiency; which may be expected in those with higher reserves (i.e. better functional integrity of neural networks).

An acute bout of exercise may promote a transient improvement in neural compensation or efficiency to improve executive function (Hyodo et al., 2012), but the extent to which neural activation is altered and executive function is improved may depend on the underlying cognitive reserves. There is currently insufficient evidence in the literature to inform a directional hypothesis as to if neural activation will be greater (i.e. compensatory) or reduced (i.e. more efficient) in response to acute exercise in those with a lowered initial reserve state, however the change was hypothesized to be associated with change in executive function.

Unfortunately, the state of the system (i.e. level of reserves) cannot be assumed by chronological age as many other factors such as health status, fitness, and genetic factors may affect cognitive reserves and executive function, as well as the relation between exercise and executive function. Others have controlled for such individual factors in the relation between exercise and executive function, but this limits our ability to understand precisely how they affect the relationship of interest. A meaningful measure that is associated with individual factors (e.g. health status, age, fitness, genetics) and can account for individual variability in response to acute exercise is needed. Here we propose parasympathetic nervous system input (herein vagal tone) as an innovative

moderator that may account for individual variability in the exercise and executive function relationship.

Vagal tone, as assessed through heart rate variability, is a proxy for the integration of brain regions that guide self-regulation, including executive function and emotional regulation (Thayer, Åhs, Fredrikson, Sollers, & Wager, 2012). Vagal tone is influenced by numerous individual factors such as fitness (Rossy & Thayer, 1998), age, sex, gender, circadian rhythms, sleep, alcohol intake, smoking, anthropometric measures, stress, and blood pressure (reviewed in Laborde, Mosley, & Thayer, 2017). Further, vagal tone is associated with measures of executive function in young (Gillie & Thayer, 2014; Hansen, Johnsen, & Thayer, 2003; Williams, Thayer, & Koenig, 2016) and older (Mahinrad et al., 2016) adults. Active older adults have higher vagal tone compared to sedentary older adults (Albinet, Boucard, Bouquet, & Audiffren, 2010) and importantly, vagal tone increases in response to chronic exercise and the increase is associated with improved executive function (Albinet, Abou-Dest, André, & Audiffren, 2016). This suggests that vagal tone may have a unique capability to provide a holistic measure of the physiological system, which could indirectly be a measure of cognitive reserves, and importantly if implicated in the magnitude of response from acute exercise, vagal tone may serve as a target for exercise interventions aimed at improving executive function. In addition, vagal tone shares a relation with BDNF (Egan et al., 2003; Griffioen et al., 2012; Wan et al., 2014; Yang et al., 2010) and neural activation (Lane et al., 2009; Sakaki et

al., 2016; Thayer, 2009) and as such may interact with exercise to predict the magnitude of change in BDNF and neural activation in response to acute exercise.

Specific Aims and Hypotheses

Given the current evidence, the following specific aims will be tested:

Aim 1. To investigate the effect of exercise on BDNF and determine the role of change in BDNF as a predictor of change in executive function in response to exercise.

The *working hypothesis* is that in response to acute exercise, BDNF will increase and the magnitude of increase will predict change in executive function.

Aim 2. To investigate the effect of exercise on neural activation and determine the role of change in neural activation as a predictor of change in executive function in response to exercise.

The *working hypothesis* is that in response to acute exercise, neural activation will change and the change in neural activation will predict change in executive function.

Aim 3. To investigate differences in change in BDNF, change in neural activation, and change in executive function in response to exercise as a function of level of vagal tone.

The *working hypothesis* is that those with low vagal tone will have greater changes in BDNF, neural activation, and executive function in response to exercise compared to those with high vagal tone.

CHAPTER II

EXTENDED LITERATURE REVIEW

Executive Function

The first known natural case-study recognizing the importance of the frontal lobe in behavior regulation was published in 1848 on Phineas Gage. After surviving an occupational accident, Phineas Gage had a hole through his frontal lobe, essentially an isolated ablation, with notable changes to his behavior such as being disorganized and impulsive (Guidotti, 2012). What began in the 19th century as recognizing the importance of the frontal lobe in regulating behavior, has now progressed to the understanding that the frontal lobe, in addition to other brain regions, is necessary for performance of core executive functions, including inhibitory control, set switching, and updating of working memory, implicated in effortful control of behavior (Diamond, 2013; Miyake & Friedman, 2012; Miyake et al., 2000). Inhibitory control refers to the ability to suppress a dominant response in favor of performing a subdominant response (Rothbart, Ellis, Rueda, & Posner, 2003). A classic example of inhibitory control is the Stroop Color-Word Test which presents color words (e.g. red, blue, green) printed in a different color ink (i.e. the word is “red”, but the ink is blue). The dominant response is to read the word; thus, it needs to be inhibited in order to respond correctly to the color of the ink (Stroop, 1935).

The second core executive function is set shifting, also known as cognitive flexibility. Set shifting involves the disengagement of an irrelevant mental set and the active engagement of the relevant mental set. For example, in a category set switching test, two sets of categorical instructions are learned (e.g. A: decide whether something is alive or not alive; B: decide whether something is larger or smaller than a soccer ball) and a cueing symbol identifies the relevant mental set. The ability to shift between sets of instructions responding only to the relevant mental set is assessed through switching cost, or the difference in reaction time during shift and non-shift trials (Friedman, Nessler, Johnson, Ritter, & Bersick, 2008).

The third core executive function is updating of working memory, which is the ability to manipulate contents in temporary, or working, memory. For example, two letters are presented (e.g. B and J), the participant is told to mentally shift each letter forward in the alphabet, and after a brief pause a probe is presented and they decide if it matches the updated letters (e.g. C and K; Chee, 2004; Slutsky et al., 2017). This involves temporal tagging, such that there is a need to keep track of new and old information separately, which is reliant on the ability to update working memory (Smith & Jonides, 1997).

The unity and diversity of the three core executive functions has been debated and thoroughly investigated (Diamond, 2013; Friedman et al., 2008; Miyake & Friedman, 2012; Miyake et al., 2000), with an important consensus that although there is overlap with these functions, they are separable constructs

(Miyake & Friedman, 2012; Miyake et al., 2000). In a confirmatory factor analysis, inhibitory control, shifting, and updating of working memory all load onto a common executive function latent variable, which correlates perfectly with inhibitory control, however shifting and updating of working memory capture unique variance (Miyake & Friedman, 2012). Given that these constructs share some overlap, declines in any of the constructs might be expected to coincide with or affect declines in other executive functions and have the potential to impact other cognitive functions, such as memory.

Aging and Executive Function

Executive functions decline during normal aging (Lachman, Agrigoroaei, Tun, & Weaver, 2014) and have been suggested to mediate age-related cognitive decline (Salthouse, Atkinson, & Berish, 2003). In older adults, declines in executive function are apparent about three years prior to declines in memory (Carlson et al., 2008), which suggests when older adults are beginning to show signs of memory decline, their executive function has most likely already declined. In further support, although decline in memory is a major symptom of Alzheimer's disease, inhibitory control, not memory, is predictive of $A\beta_{42}$/Tau protein level in asymptomatic preclinical Alzheimer's disease (Harrington et al., 2013), suggesting an early deficit in executive function that eventually affects memory. One potential explanation for this temporal sequence is that memory is reliant on frontal-striatal pathways, involved in executive function, for source information and temporal details (Buckner, 2004); the frontal-striatal pathway is

negatively affected by aging which affects executive function and has downstream effects on memory.

Declines in executive function during older adulthood impact health-related quality of life (Davis, Marra, Najafzadeh, & Liu-Ambrose, 2010; Forte et al., 2015). This relationship appears to be affected by type of executive function, such that inhibitory control is associated with mental health-related quality of life and shifting is associated with physical health-related quality of life (Forte et al., 2015). In addition, longitudinal change in executive function is associated with change in functional ability, as measured through activities of daily living (Tomaszewski Farias et al., 2009), which may in-turn affect quality of life. Of importance, older adults with poor executive function are more than twice as likely to die before those with better executive function (Peng et al., 2016; Vu, Dean, Mwamburi, Au, & Qiu, 2013). This current evidence suggests that there is a need to prevent declines in executive function to potentially prevent further declines in other cognitive functions (e.g. memory) as well as declines in the health and well-being of older adults.

Exercise and Executive Function

A promising approach for prevention of declines in executive function is through physical exercise. In a recent meta-analysis, the effect of moderate intensity acute exercise on executive function was small, but positive for time-dependent (e.g. reaction time; g=0.35) and accuracy (g=.20) measures (Ludyga et al., 2016). Although there was no overall difference between time-dependent

and accuracy measures, only the time-dependent measures were sensitive to differences between age groups. When considering only older adults, the effect of exercise on time-dependent executive function measures was positive and of moderate-size (*g*=0.67), which was similar to the effect for preadolescent children (*g*=0.54). The effect for older adults was greater than young adults and adolescents, which supports the idea that during vulnerable developmental periods (i.e. childhood, older adulthood), the benefit of exercise is greater than at other timepoints across the lifespan (Kramer et al., 1999).

These findings are not consistent with a previous meta-analysis finding age moderated the effect of acute exercise on cognition, such that larger effects were found for adolescents, adults, and older adults and smaller effects for children and young adults (Chang, Labban, Gapin, & Etnier, 2012). However, the latter meta-analysis combined all areas of cognition rather than investigating executive function per se and still supports older adults having a larger effect compared to other age groups. In addition, Chang et al. (2012) found type of task moderated the benefit from acute exercise and benefits of executive function were larger compared to the benefits of information processing, reaction time, and memory. Taken together, there is substantial evidence in support of acute exercise benefitting executive function in older adults.

With many bouts of acute exercise, through chronic exercise training, these transient benefits in executive function appear to become adaptations to exercise, benefiting executive function at rest. Colcombe & Kramer (2003)

conducted a meta-analysis on 18 exercise intervention studies with sedentary older adults and found that chronic exercise benefitted overall cognition (g=0.16), with the greatest effect on executive function (g=0.68). Further, older-old adults (66-80 years) had greater benefits compared to younger-old adults (55-65 years; Colcombe & Kramer, 2003); providing further support for greater effects of exercise on executive function seen during vulnerable developmental periods. These findings were complementary to an updated meta-analysis showing an overall benefit of exercise interventions on cognition for older adults (SMD=0.29), however in contradiction to the previous meta-analysis, the specific effect on executive function was similar to overall cognition (SMD=0.34; Northey et al., 2017). The discrepancy here may be due to study inclusion, with the later meta-analysis doubling the number of including studies (36 versus 18); however, and of importance, in the growing field of exercise and cognition there are consistent findings of a benefit of chronic exercise for older adults on overall cognition and executive function.

One inconsistency of studies that are designed to assess the relationship between exercise and executive function is with task selection. Although task selection should be grounded in neuropsychological research (Etnier & Chang, 2009), the use of multiple tasks to assess executive function should also be considered (Miyake & Friedman, 2012; Miyake et al., 2000). Out of 40 recently reviewed acute exercise and executive function studies, 24 used measures of inhibitory control, 9 used measures of shifting, 2 used measures of working

memory, and 5 used measures of at least two of the three core executive functions (Ludyga et al., 2016). Although the specific aspect of executive function tested has not been shown to moderate the acute exercise and executive function relationship (Ludyga et al., 2016), with more than twice as many studies using inhibitory control compared to working memory or shifting, this remains unclear.

Inhibitory control is perfectly correlated with the common executive function latent factor, made up of all three core components (Miyake & Friedman, 2012), and therefore if testing time is restricted it seems appropriate to use a measure of inhibitory control to assess executive function. However, that limits the ability to statistically represent the unique variance of shifting and updating, suggested to reflect flexibility and information processing, that might be affected by exercise (Miyake & Friedman, 2012). Future research needs to consider using multiple tasks to assess the effect of exercise on executive function given the unity and diversity of each of these constructs representing executive function (Miyake & Friedman, 2012; Miyake et al., 2000).

The meta-analytic evidence is clear that exercise, both acute and chronic, benefits executive function in older adults, however the mechanisms that underlie how exercise affects executive function remains unclear. Although early meta-analyses suggest mechanisms like neurotrophic expression, neural activation, cerebral blood flow, and brain structure (Colcombe & Kramer, 2003; Etnier et al., 1997), as potential mediators in the relationship it wasn't until recently that many

people began exploring the specific effects. Assessing blood biomarkers has become more readily accessible with the price of enzyme-linked immunosorbent assays being markedly reduced over the past couple of decades. One potential blood biomarker that appears to be a candidate mechanism for the effects of exercise on cognition is brain derived neurotrophic factor (BDNF). In addition, although functional magnetic resonance imaging (fMRI) studies are expensive, the ability to use this technology to indirectly measure neural activation, through the blood oxygen level-dependent (BOLD) signal, has been critical to the advancement of exercise and cognition research. As such, I will now discuss the possible mechanisms of BDNF and neural activation in the relationship of exercise and executive function.

Brain-Derived Neurotrophic Factor

Aging is associated with cognitive declines and these declines are associated with decreased gray matter volume cross-sectionally (Erickson et al., 2010) and throughout aging (Gorbach et al., 2017; Kramer & Erickson, 2007). The reduction in gray matter volume reflects declines in dendritic spine density, rather than the long theorized age-related loss of neurons (Dickstein, Weaver, Luebke, & Hof, 2013; Page et al., 2002). Given these morphological changes, it is of interest to identify factors that positively impact dendritic architecture and may be expected to counter the age-related declines in brain structure and cognitive function. BDNF is a protein that is highly expressed in the hippocampus and prefrontal cortex (Pezawas et al., 2004), is associated with

gray matter volume (Erickson et al., 2010; Hashimoto et al., 2016), and is critical for maintenance of dendritic spines (Chapleau, Carlo, Larimore, & Pozzo-Miller, 2008; Kellner et al., 2014). BDNF decreases with age, however increasing BDNF level during older adulthood is associated with positive outcomes of brain structure (Erickson et al., 2011) and executive function (Leckie et al., 2014). BDNF signaling is associated with downstream effects on synaptic plasticity, dendritic spine density, neurogenesis, and cell survival and as such is a candidate biomarker to intervene on age-related declines. I will provide a brief overview (reviewed in Cunha, 2010; Marosi & Mattson, 2014; Slutsky & Etnier, 2016; Tejeda & Díaz-Guerra, 2017) of the BDNF cell signaling pathways, along with the major downstream effects.

BDNF has two major isoforms; mature BDNF (mBDNF) and pro BDNF (proBDNF). There is an additional form of the pre-proBDNF, however that is intracellularly cleaved to proBDNF. ProBDNF has a molecular weight of 32kDa and is packaged into two types of vesicles and either intracellularly cleaved to mBDNF (molecular weight of 14kDA) with furin or secreted locally in response to neuronal excitatory signaling. During excitatory signaling, glutamate is released from the presynaptic terminal and activates post-synaptic receptors, a-amino-3-hydroxy-5-methyl-4isoxazoleprpionic acid receptor (AMPAR) and n-methyl-d-aspartate receptor (NMDAR). These receptors elicit a major influx of calcium, which triggers a further intracellular release of calcium from the endoplasmic reticulum. The increased intracellular calcium signals the release of BDNF

(primarily proBDNF) outside of the cell. Once outside of the cell, proBDNF is either cleaved to mBDNF by tissue plasminogen activator (tPA) or binds to the receptor p75 neurotrophin receptor (p75ntr). If cleaved, mBDNF can either bind to tropomyosin-related kinase B (TrkB), or p75ntr. TrkB is a high affinity receptor for mBDNF and a low affinity receptor for proBDNF, whereas p75ntr has a high affinity for proBDNF and low affinity for mBDNF.

There is a consensus on the downstream signaling pathways in the literature (Cunha, 2010; Marosi & Mattson, 2014; Slutsky & Etnier, 2016; Tejeda & Díaz-Guerra, 2017) and as such I will describe the major signaling pathways associated with TrkB and p75ntr. The binding of mBDNF to TrkB results in dimerization in the autophosphorylation of tyrosine residues. Downstream signaling through IRS ½-PI3K-Akt and Shc-Grb2-SOS-Ras-MEK-ERK results in increased CREB activity in the nucleus and BDNF transcription. Further, TrkB increases PLCγ activity, which increases intracellular calcium release which can further lead to downstream CREB activity, as well as release of proBDNF from the cell. The calcium-mediated pathways are associated with synaptic and neuronal plasticity, while the Shc pathways are associated with dendritic spine density and cell differentiation. mBDNF, through TrkB, has an indirect effect on cell survival, such that Akt downregulates BAD which is normally associated with apoptosis. In contrast, the binding of proBDNF to TrkB does not result in downstream signaling pathways and the binding of proBDNF or mBDNF to p75ntr results in pro-apoptotic pathways through JNK. The balance of the two

pathways appears critical to balance neuronal function, as seen in other antagonizing pathways throughout the central and peripheral nervous system (Marosi & Mattson, 2014).

BDNF and Exercise

There is sufficient evidence to suggest that BDNF is associated with dendritic spine density and synaptic plasticity, as well as neuroprotection and neurogenesis (reviewed in Marosi & Mattson, 2014), therefore, increasing BDNF in older adulthood may counter some expected age-related changes in the brain and prevent declines in cognitive function. The response of BDNF to acute exercise has been recently meta-analytically reviewed. Although findings have inconsistencies, after controlling for publication bias the effect of acute exercise on BDNF has an effect size of 0.4 (Dinoff et al., 2017). Important findings in this response were that duration of the exercise should be at least 30 minutes when trying to increase BDNF, there may be a higher response in higher fit individuals, and there may be an effect of sex. These findings note the importance of study design when investigating exercise and BDNF.

Following exercise, BDNF in rodents has been shown to specifically increase within the hippocampus (Berchtold et al., 2001), the prefrontal cortex (Geng et al., 2013) and the amygdala (Liu et al., 2009). This may suggest that exercise affects hippocampal-dependent cognition (e.g. spatial memory), executive function, and emotion/affect through BDNF. There is some empirical support for the response of BDNF following exercise in humans to be associated

with improved executive function in young adults and older adults. Hwang et al. (2016) investigated the effects of high-intensity acute exercise on BDNF and executive function in young adults. Following exercise, there was a significant increase in BDNF and an improvement in inhibitory control and set shifting. Further, the increase in BDNF from pre- to post-exercise was associated with the improvement in set shifting. This latter finding was contrary to that of a recent review that summarized seven articles, four of which examined executive function, and of the four with executive function there was no support for increases in BDNF being associated with increases in executive function (Piepmeier & Etnier, 2015). It has been suggested that there are numerous confounding factors in this relationship such as BDNF genotype, age, health status, lifestyle behaviors, and fitness (Hwang et al., 2016), as well as discrepancies in study design (Piepmeier & Etnier, 2015), that may account for some of the variability in results.

To date, there is only one study that has examined the effects of acute exercise on BDNF and executive function in older adults. Håkansson et al. (2017) found that following a 35-minute exergame session, BDNF was significantly increased and working memory was improved. Importantly, in a subgroup analysis separating groups into tertiles based on baseline BDNF, the lowest tertile group showed an association between increase in BDNF and increase in working memory. Given this is the only study with older adults assessing this relation, along with lower BDNF being associated with negative

outcomes (i.e. reduced cognitive function, fitness, and health), this may suggest that debilitated older adults may rely on bioenergetic changes in the brain (i.e. increase in BDNF signaling) in order to improve cognition in response to exercise.

Chronic exercise also affects BDNF, but instead of transiently, it is affected at the basal level. After one-year of exercise training for sedentary older adults, BDNF was significant increased and associated with greater hippocampal size (Erickson et al., 2011). Further, in a similar study BDNF was increased following training and the increase was shown to mediate the benefits achieved in set shifting (Leckie et al., 2014). Change in BDNF mediated the relationship between chronic exercise and executive function for the overall sample, but when further investigating age subgroups, it was found the effects were specific to the older-old group (>71 years). Like the acute study with older adults outlined above (Håkansson et al., 2017), this specificity for older-old adults may suggest that those who have declined through aging may attain greater benefits. Although empirical evidence supports BDNF as a potential mediator of exercise and executive function, there is a need for further investigation and this complex relationship is unlikely mediated through a single mechanism. As such, I will now turn my attention toward neural activation, another promising mediator in the relationship of exercise and executive function.

Neural Activation

The blood-oxygen-level dependent (BOLD) signal is a proxy signal, measured with fMRI, to infer neural activation. fMRI is sensitive to changes in deoxyhemoglobin (deoxyHb) because of its strong paramagnetic properties from four unpaired hydrogen ions at the iron center of the Hb. When neural activity increases, there is a response, termed functional hyperemia, in which cerebral blood flow locally increases. The increase in cerebral blood flow without an equivalent increase in the cerebral metabolic rate of oxygen, results in a higher ratio of oxyhemoglobin(oxyHb): deoxyHb in the venous side of the tissue. This reduction in deoxyHb distorts the magnetic field in and around the vessel, which increases the T2*, thus resulting in greater fMRI (BOLD) signal. This process has been thoroughly reviewed and theoretically is how we can infer neural activity from the BOLD signal change (Buxton, 2013; Hillman, 2014). An important finding in fMRI research was from Logothetis, Pauls, Augath, Trinath, & Oeltermann (2001) investigating the relationships amongst multi-unit activity, local field potentials, and the BOLD signal. Multi-unit activity was assessed within a couple hundred microns from the electrode needle tip, local field processing was assessed within a few millimeters of the tip, and BOLD signal was collected with fMRI. They found that multi-unit activity was reflected in spiking, which is thought to be a representation of neural activity output, whereas local-field potential had elevated signal throughout the stimuli presentation suggesting a summation of neural activity from the surrounding region and associated with

neural input, rather than output. Importantly, the BOLD signal seemed to reliably model the local field potential suggesting that the BOLD signal reflects a summation of neural activity in the area and is sensitive to increase activation during the duration of stimuli. Further they found an initial dip in BOLD signal, which may represent an initial increase in deoxyhemoglobin with increased cerebral metabolic rate prior to functional hyperemia. This hemodynamic response function is modelled in numerous fMRI studies and these findings furthered our understanding of what we are measuring with the BOLD signal.

Neural Activation and Executive Function

There is continuous BOLD signal activity, as the brain never truly rests, however the patterns of neural activation are organized in large-scale networks and are similar both with and without the presence of a task. The common saying that stems from Hebbian theory (Hebb, 1949), “those that fire together, wire together,” is reflecting similar timeseries of BOLD signal data. Although much work has gone into resting-state fMRI (patterns of neural activation without a task), here I will focus on task-based fMRI. Similar to the behavioral research on executive function, there is an understanding of unity and diversity of executive function tasks with respect to neural activation patterns. Niendam et al. (2012) meta-analytically reviewed 193 fMRI studies, which included almost 3000 subjects, and found a similar cognitive control network across all executive functions and activation in some brain regions that were task-specific. Inhibitory control, shifting, and updating of working memory all included the dorsolateral

prefrontal cortex, anterior cingulate cortex, superior and inferior parietal lobe, frontopolar cortex, premotor cortex, occipital (Broadmann area [BA] 19), and temporal (BA 13) regions. Shifting had unique activation in the orbitofrontal cortex and shifting and updating of working memory shared additional activation in the cingulate (BA 24) and temporal region (BA 37). These patterns mimic the behavioral work in executive function such that all three functions share overlap, however shifting and updating appear to have unique characteristics beyond inhibitory control (Miyake and Friedman, 2008). Given this cognitive control network is associated with executive function tasks, disruptions to this network would be expected to affect performance.

In support of this, Fjell et al. (2017) investigated the effects of changes in brain structure and function relative to changes in executive function over a 3-year period in young and older adults. Older adults declined in executive function, above and beyond changes in other cognitive processes (e.g. processing speed), and the decline in executive function was associated with changes in structural and functional connectivity. A major finding of this study was that 82.5% of the declines in executive function were explained by changes in structural connectivity. The magnitude of variance in change in executive function that was accounted for by change in structural connectivity suggests support for the disconnected brain hypothesis of aging (Bennett & Madden, 2014).

Although change in structural connectivity appears to be a major predictor of change in executive function, aging is also associated with decreases in cerebral blood flow and cerebral metabolism of glucose and oxygen (Chen, Rosas, & Salat, 2011). These latter insults with aging may affect neural activation and thus be reflected in the BOLD signal. Multiple healthy, aging brain theories have been suggested (for review see Sala-Llonch, Bartres-Faz, & Junque, 2015), but I will be discussing three: The Hemispheric Asymmetry Reduction in Old Adults (HAROLD) model (Cabeza, 2002); The Compensation-Related Utilization of Neural Circuits Hypothesis (CRUNCH; Reuter-Lorenz & Cappell, 2008); and The Posterior-Anterior Shift with Aging (PASA; Davis, Dennis, Daselaar, Fleck, & Cabeza, 2008). The HAROLD model suggests older adults compensate for neural insults by activating bilateral regions, rather than lateralized activation as seen in younger adults (Cabeza, 2002). The CRUNCH model suggests there is a greater level of neural activation in older adults compared to younger adults during a low task demand, but as demands increase older adults will experience a ceiling-effect and have reduced performance and neural activation as compared to young adults (Reuter-Lorenz & Cappell, 2008). Finally, the PASA model suggests that older adults will have less neural activation in posterior (e.g. occipital) regions and increases in frontal activity, regardless of task difficulty (Davis et al., 2008). All three of these models have support in the literature, but importantly some studies have found their data represents multiple theories (Carp, Park, Polk, & Park, 2011). This may suggest the importance to have a-

priori hypotheses, but also perform exploratory analyses to better understand healthy aging of the brain alongside age-related declines in executive function.

Neural Activation and Exercise

Some level of brain aging appears to be inevitable, but the onset and slope of the aging effects may be altered with lifestyle interventions, such as exercise. Both acute and chronic exercise have shown to affect neural activation, however the evidence of neural activation changes in response to exercise being associated with executive function is limited. Two recent studies have assessed the effects of acute exercise on resting-state connectivity and have found that in young adults had greater connectivity in sensorimotor, auditory, and subcortical networks (Rajab et al., 2014) and older adults had greater salience network connectivity in the hippocampus, amygdala, and middle temporal gyrus (Alfini et al., 2018). Although these findings are with resting-state functional connectivity, rather than neural activation in response to a task, they still provide support that a single bout of exercise can induce transient changes in the brain, similar to that of blood biomarkers. These effects of resting-state are corroborated by use of functional near infrared spectroscopy (fNIRS) and fMRI with task-based designs.

Early studies used functional near infrared spectroscopy (fNIRS) to assess the effects of acute exercise on cognition. Following acute exercise, increases in neural activation were shown in the prefrontal cortex in young adults and the frontal polar area in older adults, as assessed by fNIRS, which was related to improved executive function performance (Hyodo et al., 2012; Yanagisawa et al.,

2010). Although fNIRS provides meaningful information as to the relationship between acute exercise and neural activation, the spatial resolution is limited compared to functional magnetic resonance imaging (fMRI), which limits the conclusions that can be drawn from specific brain regions (Scarapicchia, Brown, Mayo, & Gawryluk, 2017).

Cognitive Reserve Theory

The cognitive reserve theory was initially proposed as including two types of reserves: brain and cognitive (Stern, 2002). Brain reserves referred to the brain structures and suggested that those with greater brain volume were more resilient to the onset of cognitive declines (Katzman, 2012). Brain reserves was suggested as a threshold model (Satz, 1993), such that once decline in brain structure reached a critical point, decline in cognitive performance became evident. Although the critical point would not be expected to be a static threshold, it may interact with the level of compensation—or cognitive reserves. Cognitive reserves is an adaptable model that describes the constant attempt at coping with changes to the passive model (e.g. decrease in brain volume or brain pathology) through improvements in prior neural processes or compensation through additional processing (Stern, 2002). Having a significant amount of brain pathology with no signs or symptoms of declines or disease is an example of the balance between the passive and active models.

Cognitive reserves have been quantified through level of education, occupational status, and engagement in cognitively stimulating activities, all

suggested as positive predictors of cognitive function during older adulthood (Opdebeeck, Martyr, & Clare, 2016). Interestingly, higher level of education and complex middle-age occupations were associated with a reduction in risk of cognitive impairment and being socially-engaged during later life reduced the risk of moving from slightly impaired to moderately/severely impaired (Marioni, Valenzuela, van den Hout, Brayne, & Matthews, 2012). Of importance, physical activity has also been considered in the model of cognitive reserves as low physical activity is associated with a higher risk for dementia and, with regard to the passive model, physical activity is associated with total brain and hippocampal volumes (Tan et al., 2017).

Although much of the original theory has been supported in the literature, there were some major flaws that were brought up and updated in a recent review (Stern, 2009). A major theoretical flaw with brain and cognitive reserves is the circular reasoning. For example, higher IQ and cognitively engaging environments are suggested to yield higher cognitive reserves, however there is empirical evidence for IQ and stimulating environments being associated with brain volume (Kesler, Adams, Blasey, & Bigler, 2003; van Praag, Kempermann, & Gage, 2000; Van Praag, Kempermann, & Gage, 1999; Willerman, Schultz, Neal Rutledge, & Bigler, 1991). In the updated theoretical paper, Stern described the need to understand the mechanisms of cognitive reserves by investigating the interactions of genetics and lifestyle factors on brain structure and pathology,

while also accounting for the ability to compensate for age-related declines or pathological onset of brain structure or function.

The updated cognitive reserve theory includes two major components, neural reserve and neural compensation (Stern, 2009) that interact and affect cognitive processing in older adulthood. Stern (2009) defines neural reserves as individual variability in neural efficiency (e.g. less activation with equivalent or superior performance), capacity (e.g. more activation with superior performance), or flexibility (e.g. alternative strategies), within brain networks that are needed for cognitive processing in the healthy brain. Although this extends across different levels of cognitive processing (efficiency, capacity, flexibility), individuals with higher processing ability in any of the three domains would be expected to be able to cope with brain pathology more so than those with less neural reserves.

Neural compensation is similar to neural reserves; however, it is specific to a pathological brain. With brain volume loss or disruptions in brain networks, in order to maintain or improve performance, individuals need to compensate by using alternative brain structures or networks that are not otherwise expected in healthy brains. Although neural compensation may be of interest between groups of people (e.g. healthy vs diseased), within a healthy aging population the focus would be placed on neural reserves.

Acute exercise improves cognitive performance in older adults and one proposed mechanism is that the benefits are achieved through the effect of exercise on neural activation, which places focus on the efficiency and/or

capacity aspects of neural reserves. Although individual variability in cognitive performance and neural activation would be expected, perhaps those with reduced reserves have a greater window for acute improvement, thus would have a greater effect from exercise on neural activation and performance than someone with higher reserves.

Vagal Tone

Vagal tone can be operationally defined as the summed activity of both the nucleus ambiguous and dorsal motor nucleus of the vagus (Porges, 2007). Although this would account for all of the activity of the vagus, we know very little about the dorsal motor nucleus and therefore vagal tone is usually suggested to be indexed by respiratory sinus arrhythmia (RSA), high frequency heart rate variability (HF-HRV), and/or the root mean squared successive differences (RMSSD); which are indices of the myelinated vagus from the nucleus ambiguous. Although it appears that the dorsal motor nucleus has little input on the relationship, we know that HRV indices are associated with parasympathetic, but not sympathetic nervous system, and do not account for entire vagal tone. To model the relationship of vagal tone, sympathetic nervous system input, and RSA, many have used atropine to block acetylcholine, thus depressing vagal input or atenolol, a beta-blocker depressing sympathetic input (Porges, 2007). Dellinger, Mckiernan, Koritz, & Richardson (1987) showed a dose-response effect of atropine on RSA, however RSA is not affected by atenolol (Grippo, Lamb, Carter, & Porges, 2007). Similar reports of the effects of atropine and

atenolol on RMSSD and HF-HRV (Després, Veissier, & Boissy, 2002) support these three measures as reasonable proxy measure for vagal tone. Quantifying vagal tone allows for insight into the human system including the function of the heart, brain, and other organs. As such, many have focused their research on vagal tone with one aspect of the system or vagal tone with the integration across systems.

The neurovisceral integration theory (NIT) is based in complexity theory and comprises of 8 distinct levels of neural structures that have bidirectional relationships to underlie bottom-up and top-down processing. The goal of this model is to explain the relationships between peripheral physiology, cognitive control, and emotion/physical health. The lower levels are responsible for automatized response, while the higher levels are responsible for regulating that response. This theory was first outlined by Thayer & Lane (2000) and recently updated and thoroughly reviewed (Smith, Thayer, Khalsa, & Lane, 2017). I will explain each level first and then attempt to provide meaning to their integration. Level 1 is the most basic including intra-cardiac control, level 2 begins to integrate the heart with the ANS through sub-cortical structures such as the nucleus ambiguous to adapt to reflexes, such as the baroreflex to control heart rate and blood pressure. Level 3 begins to integrate the heart and sub-cortical brain regions with other systems such as regulation of glucose and immune system function. At level 3, all of the organs are still considered to be working separately but influenced by the same factor (i.e. vagal tone). Level 4 begins the

integration of the systems together that provide feedback to the hypothalamus which can project on lower structures to elicit behavioral responses to affect homeostasis, such as thirst, hunger and thermoregulation. The lower four levels are really involving the vagus and system control up to the level of the hypothalamus, however perceptions of the environment and physiological state are not yet integrated.

At level 5, the first level of integration with higher structures begins as the hypothalamus relays information about the rest of the body onto the amygdala, which can integrate with higher structures (i.e. medial prefrontal cortex) for cognitive performance or back down to the hypothalamus for responses to emotional stimuli. This level is really associated with the automatic response, rather than regulation per se. Level 6 is the first true level of integration for regulation where information from the environment and the present state of the body are integrated in regions such as the anterior cingulate and the insula to provide volitional action, emotion, and cognitive function. Level 7 extends from level 6, with some regions of overlap (e.g. the orbitofrontal cortex) and assesses the physiological state and environment regarding potential future needs. This takes higher order processes to include previous experiences to provide the right top-down feedback and elicit the correct response; this mainly is comprising of the default mode network. This stage allows for emotional and behavioral regulation using top-down processes. Level 8 is the top and final level of the NIT and it is responsible for amplifying regulation from level 7 with goal-directed

behavior. This level is comprised of the cognitive control network that integrates information about the present state and future expectations to make regulate behavior in a relevant way towards a specific goal. At this point, this is usually considered cognitive control rather than vagal control, but it is important to understand that each level has bidirectional integration so neural processes in the cognitive control network project down onto the amygdala, hypothalamus, and nucleus ambiguous which affect vagal tone and is the basis of understanding of the relation between vagal tone and higher-order cognitive control.

As such, high levels of stress or inflammation in the periphery are projecting upwards onto the higher levels and shutting them down to allow for more automatic responses and defensive behavior, similarly suggested in the Polyvagal Theory (Porges, 2007). Together, this helps to explain why cognitive control is associated with health and all-cause mortality and higher vagal tone is associated with greater cognitive control.

Heart rate variability (HRV), specifically vagal-mediated heart rate variability as assessed by RMSSD and HF-HRV, is a proxy for the integration of brain regions that guide self-regulation, including executive function and emotional regulation. Holzman & Bridgett (2017) presented a meta-analysis of the link between HRV and self-regulation measures finding a significant link with specific impact in young-older adulthood. Thayer and colleagues at Ohio State have provided numerous accounts of this relationship in younger adults. HRV is associated with intra-individual variability in reaction time, a measure of cognitive

control (Williams et al., 2016). After a median split, the high HRV group performed better in working memory, executive function, and think/no-think tasks (Gillie & Thayer, 2014; Hansen et al., 2003). In older adults, when split into tertiary groups, the highest group performed executive function and processing tasks better than the lowest group and had less decline in performance over a follow-up of 3-4 years (Mahinrad et al., 2016). HRV has support for being associated with cognitive performance, however there are also major implications of health affecting HRV.

HRV is influenced by individual factors such as age, sex, gender, circadian rhythms, sleep, alcohol intake, smoking, anthropometric measures, stress, and blood pressure (Laborde et al., 2017). Further evidence suggests an implication of endocrine disorders, level of glucose/insulin, cortisol, and chronic inflammation affecting HRV (Laborde et al., 2017). Given this evidence, some have investigated the relation between lifestyle factors and HRV that may affect cognition. After eating salmon for 6 months, 3x/week, older adults had increased HRV and improved task switching, and HRV correlated to vitamin D status (Hansen et al., 2014). On the opposing side, trans fat intake can reduce HRV in older adults (Soares-Miranda et al., 2012). Regarding fitness and physical activity, Rossy & Thayer (1998) were one of the first to find a relationship between fitness and HRV, even after controlling for BMI. Exercise during pregnancy is linked to better autonomic control of the fetus as well as better HRV

in infancy (May, Glaros, Yeh, Clapp, & Gustafson, 2010; May, Scholtz, Suminski, & Gustafson, 2014).

There have been two exercise interventions for sedentary older adults looking at change in HRV with change in cognition. HRV and set shifting performance increased in response to 3-months of aerobic exercise (Albinet et al., 2010). Further, in response to 5 months of exercise, HRV was increased and the degree of increase was associated with degree of increase in inhibitory control (Albinet et al., 2016). These results provide evidence for HRV to possibly be a mediator of long-term intervention programs, possibly reflecting an improved system, however as a moderator for acute or cross-sectional studies suggesting that HRV can account for many factors that are influencing cognitive performance as one robust measure rather than accounting for many individual factors.

Vagal Tone and BDNF

There is an additional interaction of importance between HRV and BDNF. BDNF as suggested above is a proposed mediator for the relationship of acute and chronic exercise. Interestingly, HRV and BDNF share a relationship in autonomic function that should not be ignored. The first animal studies to show this was with a Huntington's disease mice model having lower BDNF and higher resting heart rate than wild type, but when infused with BDNF the HR returned to the same as the wild type, suggesting an influence of BDNF on parasympathetic nervous system (Griffioen et al., 2012). Further evidence of this has been shown

by using BDNF haploinsufficient mice compared to wild type. As expected, the haploinsufficient mice had higher resting heart rates, but interestingly they did not respond to an atropine injection and responded normally to atenolol. This suggests that there is specificity of BDNF to vagal tone, rather than general ANS function. In further support, when the haploinsufficient mice were centrally infused with BDNF, they then responded normally to atropine. Further experiments demonstrated that the effect of BDNF was specific to the activation of cardio-inhibitory neurons in the nucleus ambiguous—a major integration center of the parasympathetic nervous system (Thayer et al., 2012). Though studies of a similar magnitude with humans are not possible, when evaluating Met/Met versus Val/Val genotypes, known to have reduced and increased expression of BDNF respectively (Egan et al., 2003), there was support for the influence on parasympathetic nervous system activity such that the Met/Met group had reduced HRV compared to the Val/Val group (Yang et al., 2010).

Vagal Tone and Neural Activation

Neural activation is also associated with vagal tone. The neural network that underlies vagal tone (herein central autonomic network) overlaps with the cognitive control network (both include prefrontal, anterior cingulate and orbitofrontal cortices) and may help to explain the association of vagal tone with executive function (Niendam et al., 2012; Thayer, 2009). Importantly, activity of the prefrontal cortex is associated with vagal tone (Lane et al., 2009) and the neurovisceral integration theory suggests that vagal tone can be an indicator of

functional integrity of the central autonomic network (Thayer, 2009). The latter has much support as connectivity of two critical regulatory regions (medial prefrontal and amygdala) is associated with resting vagal tone (Sakaki et al., 2016). This overlap of networks and differences in functional integrity between those with high and low vagal tone may explain why higher vagal tone is associated with better executive function compared to lower vagal tone (Hansen et al., 2003; Williams et al., 2016) and suggests vagal tone as a proxy for cognitive reserves.

Theoretical Model

The model for which this study is approaching the current gap in the literature is shown in Figure 1.

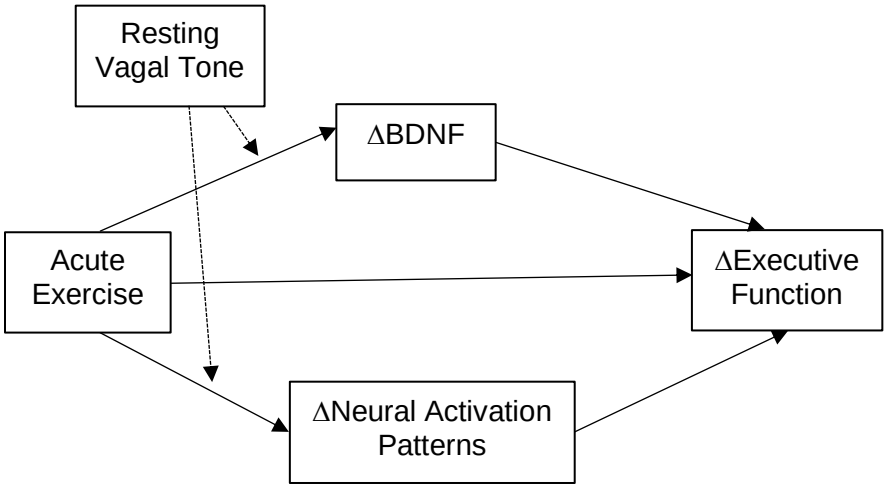

Figure 1. Illustration of the Theoretical Model

CHAPTER III

METHODS

The purpose of this study was to objectively test three specific aims: 1) To investigate the effect of exercise on BDNF and determine the role of change in BDNF as a predictor of change in executive function in response to exercise; 2) To investigate the effect of exercise on neural activation and determine the role of change in neural activation as a predictor of change in executive function in response to exercise; and 3) To investigate differences in change in BDNF, change in neural activation, and change in executive function in response to exercise, relative to rest, as a function of level of vagal tone.

Participants

Inclusion/Exclusion Criteria

Contraindications for exercise.

The American College of Sports Medicine's pre-participation health screening questionnaire was used to determine health and activity status, as well as contraindications for exercise (Riebe et al., 2015). Questions included pertinent screening information on signs, symptoms, and previous diagnoses of cardiovascular, pulmonary, metabolic, and renal disorders as well as current activity level ("Does the participant participate in planned, structured physical

activity for at least 30 minutes at a moderate intensity on at least 3 days a week, for at least the last 3 months?"). For inclusion, participants had no symptoms or previous diagnoses of cardiovascular, pulmonary, metabolic, or renal disorders; which permitted safe participation in submaximal and moderate-intensity exercise (Riebe et al., 2015).

Handedness.

Handedness was determined by the Edinburgh Handedness Inventory. Approximately 10% of the world's population is left-handed (Mandal & Dutta, 2001) and handedness can affect neural activation patterns at rest (Pool, Rehme, Eickhoff, Fink, & Grefkes, 2015) and during task performance (Cuzzocreo et al., 2009). Therefore, inclusion was limited to right-handed individuals to ensure successful recruitment (i.e., recruiting from 90% of the population rather than 10%) and to control for neural activation (e.g. hemispheric lateralization) differences.

Cognitive normality.

Normal age-related cognitive declines were of specific interest in this study and as such any older adult with significant declines relative to normal-aging were not included. Cognitive status was assessed with the Montreal Cognitive Assessment (MoCA; Nasreddine et al., 2005). The MoCA is a 30-point questionnaire that is used to assess cognitive impairment, with scores of 26-30 suggesting no impairment (Nasreddine et al., 2005). For inclusion, participants scored at least 26 points, resulting in classification as cognitively normal.

Age range.

The age range of inclusion for studies of older adults varies, however for this study only people aged 65-80 were included. Age moderates the effect of acute exercise on cognition, with recent meta-analytic findings suggesting there is a greater benefit of exercise for adults aged 50 and older (Ludyga et al., 2016) or 60 and older (Chang et al., 2012). Recent moderate intensity acute exercise studies in older adults have defined the low-end of the age range between 60-65 years with an inclusion criterion spanning across 10-30 years (Barella, Etnier, & Chang, 2010; Chang et al., 2015; Håkansson et al., 2017; Hyodo et al., 2012). This choice does not appear to have a systematic effect on the results, however it has been suggested that a broad age range (30 years) may negatively affect the ability to observe significant effects in older adults (Barella et al., 2010). Given this, older adults between the ages of 65 and 80 were recruited.

Contraindications for MRI.

There are no known risks to MR scanning for people who do not have contraindications for being exposed to a strong magnetic field (Dill, 2008). Absolute contraindications include having a heart pacemaker, metal in the head (e.g. aneurysm clips, metal ear tubes), metal in the eyes, implanted medical devices in the body (e.g. cochlear implant), implants held in by magnets (e.g. dentures), or surgery in the past 6-weeks; weighing more than 450 lbs.; or being pregnant. Other potential contraindications include situations that may put the participant at risk, but not always (e.g. tattoos are safe for MRI unless the ink

contains iron). Participants were excluded if they had any absolute contraindications to MR scanning, as well as if any potential contraindications posed a safety risk.

Normal or corrected vision.

All participants underwent a vision test with a Snellen Eye Chart to ensure visual acuity for the executive function tests during fMRI. Participants stood 10 feet away from the chart and read the lowest line that they were able to on the chart. Participants required corrective lenses if they scored less than 20/50, as they needed to be able to read size 36 font about 5 feet away. If corrective lenses were needed, they were fitted with MR safe plastic frames and lenses for their appropriate prescription or with a trial-and-error method, until 20/50 visual acuity was reached.

Colorblindness.

One of the executive function tests required the ability to distinguish the colors of red, blue, and green from each other. As such, color blindness was assessed with the Ishihara Color Test (Clark, 1924). Normal color vision was required to be included in the study.

Number of Participants to be Recruited

The number of participants needed for this study was based on an A priori power analysis and additional empirical evidence that was suggestive of the sample size needed for sufficient power. The expected effect size for the effects of acute exercise on executive function was g=0.67, based on the older adult

subgroup analysis from Ludyga et al. (2016), a meta-analysis including studies only with moderate intensity acute exercise and executive function outcomes. The power analysis with G*Power revealed n=8 to achieve a power of 0.8 in a repeated measures multivariate analysis of variance (RM-MANOVA), within factors analysis.

In addition, power was based on a recent study investigating the effects of exercise on BDNF (Håkansson et al., 2017). They found moderate intensity exercise to increase BDNF with the effect size of d=0.75. The power analysis revealed n=16 to achieve a power of 0.8 in a paired t-test to assess the effects of exercise on BDNF. Participants were able to opt-out of having blood draws and still participate in the rest of the study. Therefore, the goal was 20 participants with an expectation that up to four may opt-out of blood and we would maintain power for BDNF analyses.

Finally, power was also based on prior studies including exercise and fMRI (either task-based or resting-state). Current within-subjects design studies have included 9-17 participants (Chen et al., 2016; Kelly et al., 2017; Li et al., 2014; MacIntosh et al., 2014), whereas between-subjects designs have included total samples of 25 (Voss et al., 2016) or 50 (Metcalfe et al., 2015) subjects. Given this range, the inclusion of 20 participants, based on the other markers, was determined as sufficient to power the analyses testing the within-subjects effect of acute exercise on neural activation.

Of importance, though these independent measures were sufficiently powered, we recognize that there is not sufficient power to test the full moderated-mediation model (as shown in Figure 1). However, this does allow for testing of each specific aim in this preliminary examination of these research questions.

Study Participants

Nineteen healthy, right-handed, older adults who were cognitively normal and between the ages of 65-80 years were recruited to participate in this study. Participants were screened through email or on the phone for all major exclusion criteria and were further screened in person on day one. Out of nineteen participants who passed the email/phone screening, three were excluded because they scored less than 26 on the MoCA. The University of North Carolina at Greensboro Institutional Review Board approved the testing protocol and all participants gave written informed consent prior to participation.

Overview of Study Design

A within-subjects, counterbalanced design was used and included three testing days for each participant (Overview in Table 1). Visits included a baseline visit, an exercise visit, and a rest visit with a goal to be separated by at least 7 days, but no more than 14 days (Chu, Chen, Hung, Wang, & Chang, 2015). The three visits were scheduled at the same time of day within participants and all participants were tested in the morning between the hours of 6 am-12 pm (Chang et al., 2012). Participants were instructed to maintain normal routines the

mornings of testing, however if a meal or caffeine was part of their morning routine, they were asked to consume this 2hrs prior to testing on all days (Quintana & Heathers, 2014). A detailed description of the study design is included in Table 1.

Table 1. Overview of the Study Design

Day One	Days Two & Three
Consents and Screening (15 min)	Screening (10 min)
Questionnaires (10min)	Vision Tests (5 min)
Blood pressure, height/weight, heart rate (15 min)	MRI and resting-state fMRI (15 min)
Executive function tests (30 min)	Blood draw (Pre-condition; 5min)
Blood draw (baseline; 10 min)	Exercise or rest (counterbalanced; 30 min)
Submaximal exercise test (10 min)	Blood draw (Post-condition; 5 min)
Send home the CHAMPS (5 min)	fMRI with executive function tests and MRI (40 min)
Total time: 1 hour and 35 minutes	Total time: 1 hour and 50 minutes

MRI: magnetic resonance imaging; fMRI: functional magnetic resonance imaging

Day One

Participants were consented, completed screening forms, and had measurements of blood pressure, height and weight, and resting heart rate taken. Further, participants completed three executive function tests, had blood drawn, and completed a submaximal exercise test. During baseline executive function testing, participants needed to achieve sufficient accuracy (70%) on at least two out of three tasks to continue participation. For this reason, executive function testing preceded the blood draw to avoid an unnecessary burden to

participants as well as cost to the researcher if the participant were to be excluded from further testing.

Days Two and Three

Days two and three were counterbalanced for condition (rest, exercise) and took place at the Joint School for Nanoscience and Nanoengineering in the Gateway MRI Center. Participants completed a screening questionnaire, a vision test, and baseline structural MRI and resting-state fMRI scans. Then, participants had a pre-condition blood draw, completed the prescribed condition (exercise or rest), had a post-condition blood draw, and a post condition fMRI scan during performance of executive function tests.

Experimental Conditions

The exercise condition consisted of a 30-minute bout of cycling on the Lode Corival CPET Ergometer (Lode, Groningen, Netherlands), including a 5-minute warm-up and 25 minutes of exercise at 55-65%HRR. During exercise, watts were recorded every minute, heart rate was recorded every other minute, and RPE was recorded every 5 minutes. Heart rate was recorded from the Polar V800 monitor (previously described; Polar, USA). An educational video was played for the participants during the exercise session. The rest condition was identical to the exercise condition, regarding timing of measures and playing of a video, however instead of cycling, participants sat quietly on the bike. Watts were converted to kilocalories as a measure of work during the exercise condition (Watts*0.0146*30minutes). Average heart rate, RPE, and work were reported.

Measures of Interest

Executive Function

Executive function tests included a measure of inhibitory control, set shifting, and updating of working memory. Although this study measured the three core components of executive function (Miyake & Friedman, 2012), the order of the tests were presented in order of priority. Out of the three components, inhibitory control is the only measure that is perfectly correlated with a latent executive function factor derived by performance in all three tests (Miyake & Friedman, 2012). Therefore, it can be inferred that changes in inhibitory control can be generalized to changes in executive function. Perhaps this is why inhibition has been frequently used in acute exercise paradigms, with shifting and updating of working memory less common (Ludyga et al., 2016). Given this, inhibitory control was the primary executive function outcome for this study and was presented first. Secondary outcomes included set shifting and working memory and they were presented second and third, respectively.

The effects of exercise on cognition have been observed to have the greatest effects 11-20 minutes after exercise and small, but positive effects after a 20-minute delay (Chang et al., 2012). The time of delay from cessation of exercise to the start of the executive function tests was expected to be approximately 10-15 minutes, including the post exercise blood draw. Given that each test takes about 10 minutes to complete, the window for the transient benefits of exercise was narrow and as such the tests need to be ordered by

priority, rather than counterbalanced. Although this can introduce order effects that will limit our interpretation if there are null effects of tasks presented later in the order, it will allow for meaningful interpretation of the primary outcome.

During executive function testing on day one, participants needed to achieve 70% accuracy, over at least 30 seconds on practice trials to continue to the test trials. Practice trials timed out after two minutes; at that time the researcher explained the instructions again, confirmed the participant understood and ensured their fingers were placed correctly on the response buttons. Participants had three attempts at the practice trials before moving to the next task. If they were unable to achieve accuracy and complete the test trials for at least 2 out of three of the tasks, they were excluded from participation. Of importance, because the aims of this study were limited to the assessment of executive function in response to conditions on days 2 and 3, the baseline data was not reported.

Inhibitory control.

The Stroop Color-Word Test (SCWT) was used to measure inhibitory control (Stroop, 1935). This test presented congruent, neutral, or incongruent stimuli based on the word and the color of the word. Congruent stimuli were color words that were presented in the same ink color as the word (e.g. Red). Neutral stimuli were non-words that were presented in colored ink (e.g. XXXX). Incongruent stimuli were color words that were presented in a different color ink (e.g. Red). Participants were asked to respond to the color of the words, ignoring

the word itself. Performance in the congruent and neutral conditions are measures of speed of processing (Etnier & Chang, 2009), whereas performance in the incongruent condition is a measure of inhibition (Miyake et al., 2000; Scarpina & Tagini, 2017). The SCWT was adapted into a computerized version (Langenecker, Nielson, & Rao, 2004) with E-prime (Psychological Software Tools, Sharpsburg, PA) that is compatible with fMRI using a block-design with trial blocks for each condition (congruent, neutral, incongruent). During day one testing, participants responded on the keyboard (1=Red, 2=Blue, 3=Green) using the first three fingers of their right hand. During the fMRI, participants responded with the MR-safe serial response box (Psychological Software Tools, Sharpsburg, PA) with the same three fingers; index finger=Red, middle finger=Blue, ring finger=Green. Participants went through a series of trials to learn the correct button for each color prior to beginning the practice and test trials.

The SCWT was presented on a black background with white font, except for the stimuli which were presented in red, blue, or green. Stimuli were presented in the center of the screen in size 36 Courier New font. Throughout the test, a visual display of the correct response buttons was shown on the right of the screen. Trial block durations were 30 sec with stimuli presented for 1250 ms and an inter-stimulus interval (ISI) of 750 ms. During trial blocks, stimuli of the same condition were presented in a random order. Trial blocks were preceded

and followed by 15 sec rest periods. Each condition was presented 4 times. A visual depiction of the test is shown in Figure 2.

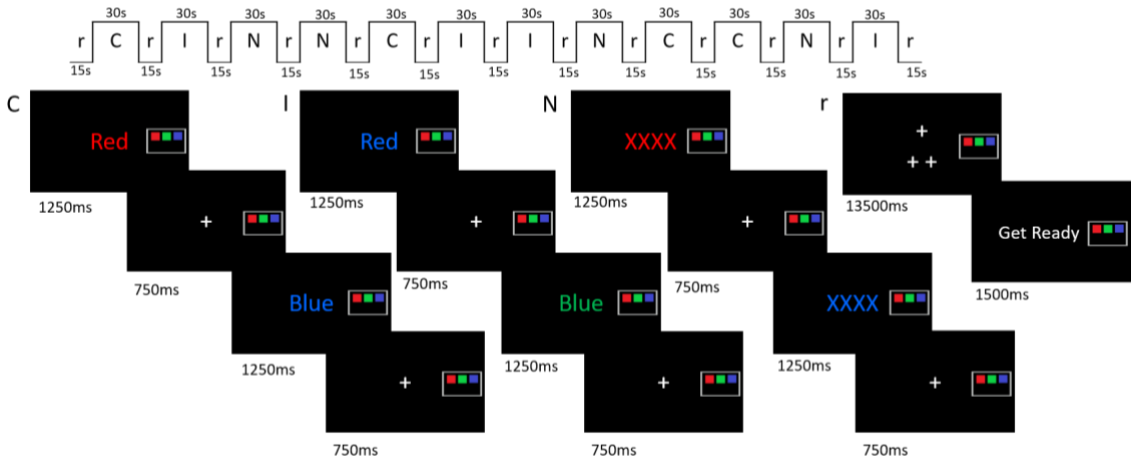

Figure 2. Depiction of the Stroop Color Word Test. Conditions include congruent (C), incongruent (I), neutral (N), and rest (r).

Time-dependent measures, not measures of accuracy, appear to be sensitive to age differences in the response to exercise (Ludyga et al., 2016). This has been suggested to reflect a ceiling effect of accuracy, whereas a greater variability is shown in time-dependent measures (Chang et al., 2015; Hyodo et al., 2012; Ludyga et al., 2016). Although there may be a greater sensitivity to time-dependent measures, interpreting reaction time without accuracy is not recommended (Scarpina & Tagini, 2017). Therefore, average reaction time during correct response trials was assessed. Measures of interest during the SCWT was reaction time for the incongruent (inhibitory control) and neutral (processing speed) trial types.

Set shifting.

An fMRI-compatible category switching paradigm, adapted from a computer test (Draheim, Hicks, & Engle, 2016), was used to assess set shifting and was presented through E-prime (Psychological Software Tools, Sharpsburg, PA). The test included two separate categories for sets of instructions, a living set and a size set. For the living set, participants saw a heart shaped-cue and decided whether the word below the cue was alive or not alive. For the size set, participants saw a soccer ball-cue and decided whether the word below the cue was larger or smaller than a soccer ball. For both sets of instructions, participants responded with their index and middle finger on buttons 1 and 2, respectively, on a keyboard during baseline testing or on the MR-safe serial response box (Psychological Software Tools, Sharpsburg, PA) during the fMRI. A correct answer with the index finger was in response to something that is alive or smaller than a soccer ball. A correct answer with the middle finger was in response to something that is not alive or larger than a soccer ball. Participants learned each set of instructions, practiced switching between the sets, and then began the test trials.

The set shifting test was presented on a black background with white font. The stimuli were presented in the center of the screen, below the cue, in size 36, Courier New font. The cue preceded the stimuli by 300 ms. Stimuli were visible until a response or up to 4.5 seconds, followed by an ISI for 300 ms of a blank black screen. Participants went through as many trials as possible during each

trial block of 30 seconds. Trial blocks consisted of a high load block, of which 70% of the trials were switching trials, and a low load block, of which 20% of the trials were switching trials (Sylvester et al., 2003). Trial blocks were preceded and followed by a rest block of 15 seconds (identical to the SCWT in Figure 2). A visual depiction of the set shifting test is shown in Figure 3. Reaction time during correct trials was assessed for switch and repeat trials during each block (Altmann, 2007; Leckie et al., 2014).

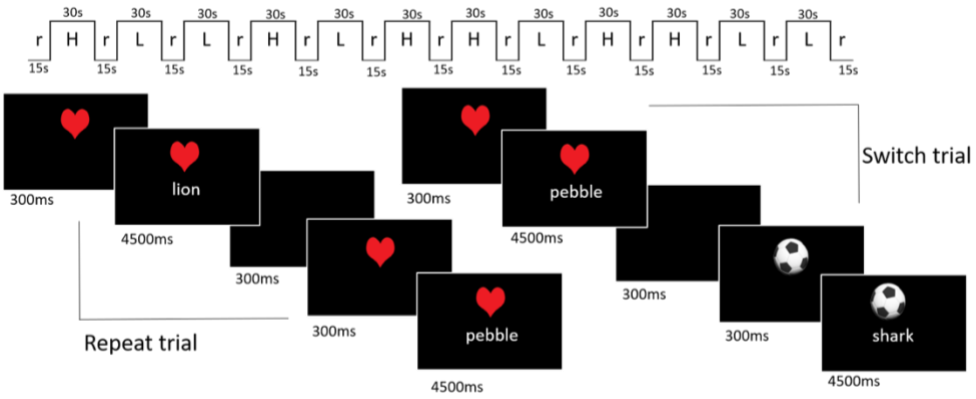

Figure 3. Depiction of the Category Switching Test. High switching load (H) has 70% switch trials and the low switching load (L) has 20% switch trials. The rest (r) condition is identical to the SCWT (Figure 2).

Updating of working memory.

An adapted fMRI compatible version of the PLUS test (Chee, 2004; Slutsky et al., 2017) was used to assess working memory and was presented with E-prime (Psychological Software Tools, Sharpsburg, PA). This test required participants to manipulate information in verbal working memory. Participants were shown two letters, separated by the presentation of a fixation cue, and

needed to mentally shift the letters forward in the alphabet to respond to the probe. For example, if B and J were shown, c and k would be correct response answers. Participants responded with their index and middle finger on buttons 1 and 2 on a keyboard during baseline testing or buttons 1 and 2 on the MR-safe serial response box (Psychological Software Tools, Sharpsburg, PA) during the fMRI. In response to the probe, participants pressed button 1 if the probe matched the mentally shifted letters or 2 if the probe did not match the mentally shifted letters. Participants went through a series of practice trials to assure an understanding of the test.

The PLUS test was presented on a black background with white text in Courier New font, size 50. The stimuli were presented for 500 ms, followed by a fixation cue for 3000 ms, a probe for 1500 ms, and an ISI for 500 ms. The probe was visible until response or up to 1500 ms. Participants went through as many trials as possible during each trial block of 30 seconds. Trial blocks alternated with 30 second rest blocks, a prolonged version of the rest block shown in Figure 2. A visual depiction of the PLUS test is shown in Figure 4. Working memory performance was measured as reaction time during correct trials.

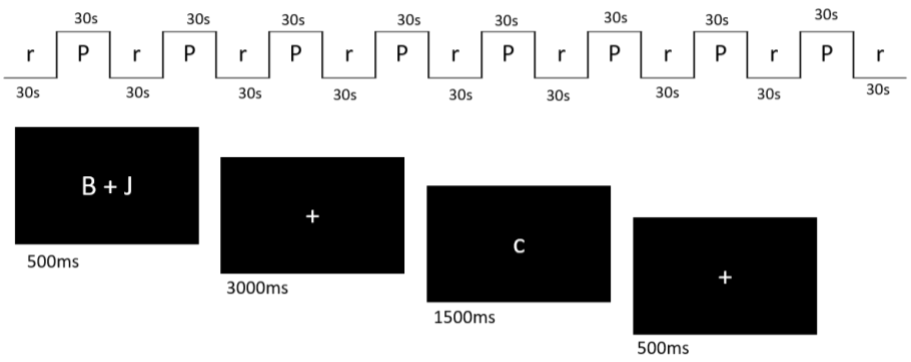

Figure 4. Depiction of the PLUS Working Memory Test. Task presented during the P blocks. The rest (r) is identical to the SCWT (Figure 2).

Brain Derived Neurotrophic Factor

Blood sample collection.

Each participant had 5 blood draws: baseline, pre-rest, post-rest, pre-exercise, and post-exercise (see Table 1). Each blood draw yielded about 20 ml of blood and the blood was processed according to the standards set forth by Polacchini et al. (2015). After the blood draw, blood samples clotted for 1 hour at room temperature and then 1 hour at 4°C. Then, samples were spun in the centrifuge at 2000*g* for 10 minutes at the temperature of 4°C. Serum was aliquoted into small storage tubes and stored in the freezer at -80°C until all samples were collected and ready for analysis.

These procedures were followed exactly for the baseline testing, as all equipment was readily available in the exercise physiology laboratory. For days two and three, the samples clotted for one hour at room temperature and then were placed on ice for the one hour of clotting at about 4°C. Once the post-

condition blood sample were placed on ice, both the pre and post-condition samples were then transported to the exercise physiology laboratory to continue with the rest of the procedure. Of importance, because the aims of this study were limited to assessment of BDNF in response to exercise and rest, the baseline samples were preserved for future analyses and we report only the findings from days 2 and three.

Quantification of BDNF.

BDNF was quantified as mature BDNF from the serum with the Aviscera-Bioscience Human BDNF sandwich enzyme-linked immunosorbent assay (ELISA) kit (Aviscera-Bioscience, Inc). This ELISA kit was recently compared to five other popular ELISA kits and it was found to have the best overall performance for mature BDNF measurement regarding intra-assay variation, inter-assay variation, detection range, sensitivity, and processing time (Polacchini et al., 2015). Importantly, the cross-reactivity with pro-BDNF was minimized, as compared to other kits (Polacchini et al., 2015), and is suggested to be less than 1% (Aviscera-Bioscience, Inc.). Aside from the preparation of the blood sample for storage (as previously explained), we followed the manufacturer instructions for the ELISA and mature BDNF was calculated using a standard curve and reported as nanograms/milliliter (ng/ml).

Vagal Tone

Heart rate, measured as R-R intervals, was collected for 8 minutes at rest on day one with a Polar H10 heart rate sensor chest strap and a Polar V800

heart rate monitor (Polar, USA). The Polar V800 heart rate monitor has been validated against electrocardiogram during an orthostatic test and was found to have less than 0.1% error rate in R-R interval detections (Giles, Draper, & Neil, 2016); a marked improvement over previous Polar heart rate monitors such as the S810 and S810i with error rates of 0.4% (Gamelin, Berthoin, & Bosquet, 2006) and 6.93% (Vanderlei, Silva, Pastre, Azevedo, & Godoy, 2008), respectively.

The collection of R-R intervals has been subjected to scrutiny in recent research, and as such the methodology for collecting R-R intervals followed the current recommendations for heart rate variability data collection (Laborde et al., 2017). Participants sat quietly with knees at a 90-degree angle, feet flat on the floor, hands resting on thighs with palms facing upwards, and eyes closed. Participants were asked to relax and to breathe normally. It is suggested to control for the time of day of heart rate assessment within and across subjects in a research study (van Eekelen, Houtveen, & Kerkhof, 2004) and as such, participant visits occurred only in the morning hours between 6 am-12 pm. Further, eating (Lu, Zou, Orr, & Chen, 1999) and consuming caffeine (Zimmermann-Viehoff et al., 2016) can affect heart rate variability measures, so participants were asked to consume any food and caffeine as part of their normal routine at least 2 hours prior to testing.

Heart rate, measured in R-R intervals, was reduced to resting heart rate and vagal tone with Kubios software (Tarvainen, Niskanen, Lipponen, Ranta-aho,

& Karjalainen, 2014). R-R interval text files were exported from the Polar heart rate data and imported into Kubios, where the data was reduced to heart rate variability metrics (e.g. time domain, frequency domain, and nonlinear metrics). A continuous window of 5 minutes of R-R intervals, from the 8 minutes of data collected, was used for data reduction. In Kubios, artifact correction is made with a threshold-based artefact correction algorithm that compares every R-R interval against a local average interval. The local average interval is not affected by single outliers, however correction can be applied to outliers if the R-R intervals differ from the local average interval by more than specific threshold (e.g. very low filter has a threshold of 0.45 seconds; Tarvainen et al., 2014). Artifact correction, even at the lowest filter (very low) in Kubios can lead to an 11% error rate in vagal tone (Laborde et al., 2017). We planned to inspect data for artifact, but not apply artifact correction (Laborde et al., 2017). However, in 9 out of 15 participants we failed to find a 5-minute continuous artifact free set of data points to select. Therefore, artifact correction was applied at the lowest filter (very low).

High-frequency power, a heart rate variability metric in the frequency domain, is a measure of vagal tone (Thayer et al., 2012) and was initially of specific interest here. This measure was chosen based on the theoretical underpinnings of the Neurovisceral Integration Theory (Thayer et al., 2012), which largely focusses on high-frequency power. In addition to high-frequency power, root mean square of successive R-R differences (RMSSD) is a measure of vagal tone that is also heavily incorporated in the neurovisceral integration

theory. RMSSD and HF power are highly correlated (Kleiger, Stein, & Bigger, 2005), however RMSSD is not influenced by respiration, whereas HF power is (Hill, Siebenbrock, Sollers, & Thayer, 2009). Therefore, both HF power and RMSSD were reduced from R-R intervals, however RMSSD was used as the measure of vagal tone.

To estimate HF power, a spectral power analysis with a Fast Fourier Transformation (FFT) was used to estimate power in the ultra-low frequency (<.003Hz), very low frequency (.0033-.04Hz), low frequency (.04-.15Hz), and high frequency (.15-.40Hz) bands (Shaffer & Ginsberg, 2017). High-frequency power was reported in terms of absolute power (ms^2) and relative power (as a % total power), as suggested by the Task force of the European Society of Cardiology the North American Society of Pacing Electrophysiology (Electrophysiology, 1996). We had planned to perform a tertiary split based on baseline vagal tone, however due to the limited sample size with vagal tone measures (n=15), a median split was used.

MRI and fMRI Procedures

Pre-condition MRI and resting-state fMRI.

Image acquisition.

All scans were performed on a 3T Siemens Magnetom TrioTim MRI scanner using a 12-channel head coil (Siemens; Erlangen, Germany). T1-weighted structural images were acquired (repetition time = 2530 ms; echo time = 2.26 ms, matrix field of view = 256 mm; flip angle=7 degrees; voxel size = 1

mm x 1 mm x 1 mm). Resting-state fMRI images, through whole-brain gradient-echo echoplanar images, were acquired (repetition time = 2000 ms; echo time = 12 ms, phase encoding direction = anterior to posterior; matrix field of view = 212 mm; flip angle=73 degrees; voxel size = 3.3 mm x 3.3 mm x 3.3 mm). For the resting-state fMRI, 48 mm slices were acquired in the transverse plane using an interleaved sequence with 183 total volumes. The first three volumes were discarded to account for scanner preparation and equilibration effects. During the structural MRI, participants were asked to be still in the scanner but told they could be awake or asleep and have their eyes open or closed. During the resting-state fMRI participants were asked to be still in the scanner, remain awake with eyes open fixated on the cross-hair in front of them (seen through a double-sided mirror), and to let their mind wander.

Data preprocessing.

Pre-condition MRI and fMRI images were not preprocessed or analyzed for this study; however, they were critical for collection of post-condition images as they habituated the participants to the environment and prepared them for the testing procedures (e.g. putting in headphones). During pilot data collection on two participants, the explanation of the pre-scan procedures took about 10 minutes prior to the pre-condition scan and 4 minutes prior to the post-condition scan; which suggests the need for a pre-condition scan in order to reduce the delay between the end of condition and the start of post-condition scans.

Post-condition MRI and task-related fMRI.

Participants viewed a screen through a double-sided mirror where the tests were projected. The executive function tests (Figures 1, 2, and 3) were presented by a ViewSonic DLP Projector (model PJD5134; ViewSonic, USA) that was connected to an HP Laptop with E-prime software. Once the participants were in the scanner, they placed their index, middle, and ring finger of their right hand on the corresponding Lumina response pad (model LS-LH; Cedrus Corporation, USA) buttons in a comfortable position by their side. Before beginning the test, correct buttons needed to be pressed to advance through the instructions and practice trials. As such, both participants and researchers were aware if the fingers were not positioned correctly and adjustments were made if necessary.

Image acquisition.

fMRI imaging for each of the three tests (SCWT, Category Switching, and PLUS) included whole-brain gradient-echo echoplanar images (repetition time = 3000 ms; echo time = 28 ms, phase encoding direction = anterior to posterior; matrix field of view = 220 mm; flip angle=78 degrees; voxel size = 2.5 mm x 2.5 mm x 2.5 mm). Slices (55mm) were acquired in the sagittal plane using an interleaved sequence. During the SCWT and Category Switching, 188 total volumes were acquired. During the PLUS test, 173 total volumes were acquired. The first three volumes were discarded to account for scanner preparation and equilibration effects.

T1- weighted structural images were acquired after the completion of all fMRI images (repetition time = 2530 ms; echo time = 2.26 ms, matrix field of view = 256 mm; flip angle= 7 degrees; voxel size = 1 mm x 1 mm x 1 mm). During the structural MRI, participants were asked to be still in the scanner but told they could be awake or asleep and have eyes open or closed.

fMRI data preprocessing.

Imaging data was preprocessed with FMRIB's Software Library (FSL; Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012) FEAT tool. The dicom images exported from the Siemens scanner were converted to nifti format with dcm2niix. Then FSL's brain extraction tool (BET) was used for brain extraction (Smith, 2002). The BET option of bias field and neck cleanup was used with a fractional intensity threshold of 0.3 (as suggested by UCLA Center for Cognitive Neuroscience). This option reduces image bias and residual neck voxels. Further preprocessing was completed as set forth by Pruim et al. (2015) with use of Independent Components Analysis-based Removal of Motion Artifacts (ICA-AROMA).

Head motion is a common problem in fMRI research and when regression methods are used for removal of motion artifact, stimulus-correlated motion is regressed out; thus, motion during the time of the test is removed. This then reduces the signal-to-noise ratio (SNR) and affects the results. Approaches to removal of motion through independent component analyses to maintain the SNR have been successful (Kundu et al., 2013; Thomas, Harshman, & Menon,

2002), however choosing components to remove is subjective and may not be the best approach. ICA-AROMA uses the spatial components, and associated time-series, derived from FSL's Multivariate Exploratory Linear Decomposition into Independent Components (MELODIC) tool to automatically identify motion-related components. ICA-AROMA selects motion components based on maximum realignment parameter correlation, edge fraction, cerebrospinal fluid fraction, and high-frequency content. When using automated motion removal about 70% of the components are typically classified as noise (Griffanti et al., 2017, 2014; Rummel et al., 2013). Motion components are usually high in number, but very small in cluster size compared to signal components that are usually low in number and large in cluster size (Griffanti et al., 2017). By identifying and removing motion-related components, ICA-AROMA is superior to typical regression methods (Friston, Williams, Howard, Frackowiak, & Turner, 1996) and preserves the SNR (Pruim et al., 2015). Thus, this is the method that was used. Each set of images went through two major steps of preprocessing. The first preprocessing step prepared the data for ICA-AROMA, the second step used the output of ICA-AROMA as the input for the first-level analyses.

Pre-ICA-AROMA.

During Pre-ICA-AROMA, four pre-processing steps were completed: 1) motion correction with FSL's motion correction tool (MCFLIRT); 2) interleaved slice-timing correction; 3) spatial smoothing with a Gaussian kernel of 6mm full-width half-maximum; 4) intensity normalization. Registration included both linear

and non-linear methods, which has been previously suggested when analyzing multiple people (Andersson, Jenkinson, & Smith, 2007; Jenkinson et al., 2012). Registration is a two step-process as functional images need to be registered to structural images and structural images need to be registered to standard space. Each subject's fMRI images were registered to their high-resolution structural image with affine registration through FSL's linear image registration tool (FLIRT). Further, structural images were registered to a standard space (MNI 152) with both FLIRT and FSL's non-linear image registration tool (FNIRT). Motion was reported as absolute and relative displacement.

A feat directory was created following the initial preprocessing step. This feat directory was used as the input for ICA-AROMA. After ICA-AROMA, text files were inspected in order to report the number of motion components that were removed from the data. Removal of components was reported as a relative measure: components removed/total components. The output of ICA-AROMA was then the input for the second step of preprocessing.

Post-ICA-AROMA.

During the second step of preprocessing (Post-ICA-AROMA), a high-pass filter was applied at 60 seconds (twice the test block). Registration was applied to the independent component maps from ICA-AROMA with the same process as the first step, using FLIRT and FNIRT. During the second step of preprocessing, first-level, or individual-level, analyses were conducted. Timing files for each executive function test were exported from E-prime and entered into the first-

level analysis in a 3-column format. This included a column of condition onset, duration, and weight for each unique condition (e.g. incongruent, congruent, and neutral) within each test Although each cognitive test is the same across individuals, the pilot data revealed that the exact timing of onset and duration varies slightly (Table 2); therefore, timing files were entered at an individual level. FMRIB's Improved Linear Model (FILM) prewhitening was also applied as it improves estimation efficiency and the data here will not violate any of the assumptions (e.g. long TRs or minimal data points). The first-level analyses resulted in estimates of main effects for each trial type compared to rest using a general linear model.

Table 2. Timing files from E-prime for Two Pilot Participants. Trial onset and duration reported in milliseconds during the SCWT incongruent condition following each condition.

Participant 1 Post-Rest		Participant 1 Post-Exercise		Participant 2 Post-Rest		Participant 2 Post-Exercise	
Onset	Duration	Onset	Duration	Onset	Duration	Onset	Duration
59.05	29.63	59.30	29.20	59.31	28.97	59.30	29.20
236.81	28.68	236.64	28.97	236.69	29.55	236.64	28.97
280.48	28.57	280.60	29.18	281.12	28.82	280.60	29.18
500.19	29.03	502.23	29.58	502.28	29.63	502.23	29.58

Head motion.

Although head motion was addressed with ICA-AROMA during preprocessing, minimizing head motion during scans was important. To reduce head motion, a strap was placed across the shoulders of the participants, foam

pads were set any open space of the head coil, and a weighted bag was placed on the forehead.

Additional Measures

Physical activity.

To assess recent physical activity, participants completed the community healthy activities model program for seniors (CHAMPS) physical activity questionnaire (Stewart et al., 2001). Moderate to vigorous physical activity (MVPA) minutes per week was scored based on previous scoring recommendations (Stewart et al., 2001). Participants reported average time spent in each moderate to vigorous activity per week in the CHAMPS as less than 1 hour (scored 0.5 hours), 1-2.5 hours (scored 1.75 hours), 3-4.5 hours (scored 3.75 hours), 5-6.5 hours (scored 5.75 hours), 7-8.5 hours (7.75 hours), or 9 or more hours (9.75 hours). Instead of converting to MET-min/week (Stewart et al., 2001), here we reported MVPA as average time in minutes to more easily be able to compare to the physical activity guidelines reported in MVPA.

Cardiovascular fitness.

The Ekblom-Bak cycle ergometer test (EB-test) was used for submaximal exercise testing (Björkman, Ekblom-Bak, Ekblom, & Ekblom, 2016). The EB-test was designed as a low-risk, time-effective test to estimate VO_2max (Ekblom-Bak, Björkman, Hellenius, & Ekblom, 2014). Recently the prediction equations have been updated (Björkman et al., 2016), have been validated for older adults, and compared to other popular submaximal exercise tests. In adults over the age of

65 (range 65-89), the EB-test explained 80% of the variance in the VO_2max test. Further, in adults ages 50-64 years, the EB-test explained 85% of the variance in VO_2max, compared to 69% explained by the Astrand-Rhyming (Björkman et al., 2016).

The EB-test was performed on the Lode Corival Recumbent Ergometer (Lode, Groningen, Netherlands) and consisted of two four-minute stages on a cycle ergometer. During the first stage, participants cycled at 60 revolutions per minute (RPMs) with a resistance of 30 watts. Heart rate was recorded every 15 seconds during the last minute and the four measures were averaged together as the heart rate for that workload. As participants moved into the second stage, the RPMs remained constant, but the resistance was increased to a self-selected workload. Participants were told to select a resistance that corresponded to a 14-15 rating of perceived exertion (RPE; Borg, 1982). VO_2max was estimated separately for women and men (shown below; Björkman et al., 2016). In the formulas below, Exp is denoting the inverse of the natural log (e^x), ΔHR is the difference of heart rate between stages one and two, and ΔPO is the difference of power (in Watts) between stages one and two (Björkman et al., 2016).

Men: $VO_2max = Exp((2.04900 - 0.00858*Age) – (0.90742*\Delta HR/\Delta PO) + (0.00178*\Delta PO) – (0.00290*HR_{stage1}))$

Women: $VO_2max = Exp((1.84390 - 0.00673*Age) – (0.62578*\Delta HR/\Delta PO) + (0.00175*\Delta PO) (0.00471*HR_{Stage1})$

The estimated VO_2max was used to prescribe the exercise intensity for the exercise condition day. Relative VO_2max was converted into 55 and 65% VO_2 Reserve (VO_2R) using the formula (example for 55%): 55% VO_2=((VO_2max-3.5ml/kg/min)*0.55) + 3.5ml/kg/min. Further, both 55% and 65% VO_2R were used to calculate the prescribed power (Watts) using the formula: (VO_2-7)*(body weight in kg)/10.8. These metabolic equations (American College of Sports Medicine, 2013) allowed for appropriate exercise prescription based on the EB-test. An additional measure of heart rate reserve (HRR) was calculated to further provide appropriate exercise prescription. Percent HRR was used as the primary method for exercise prescription, with power used as a secondary and guiding method which allowed for a faster transition from warm-up to the exercise protocol. Two participants were unable to complete the submaximal exercise test, due to the need for medical clearance prior to participation or failing to take prescribed blood pressure medications, and therefore only HRR was used to prescribe their exercise intensity.

Safety Plan

Safety of the participants throughout the study was a priority. The research team was CPR/AED certified and, if needed, could perform CPR and use an AED on participants. The research team was trained on emergency procedures for each visit.

Blood Techniques

The primary investigator (student) was a certified blood technician and was responsible for blood draws. In the case of a challenging blood draw, another certified blood technician assisted with the blood draw. All blood draw procedures followed OSHA standards to protect the participant and the research team.

MRI/fMRI Scanning

In addition to screening for participant safety for exposure to a strong magnetic field, further safety procedures were taken to protect the participants during MRI and fMRI scans. Each participant placed ear plugs in each ear with the proper insertion technique which includes rolling the ear plug into a narrow tube, reaching above or behind the head to grab the top of the ear with the contralateral arm, pulling the ear back slightly and inserting the ear plug, and finally placing a finger on the ear plug once inserted to allow for expansion inside of the ear canal (Moldex, USA). In addition, participants placed MR safe headphones on top of the ears to complete the auditory protection. This procedure allowed participants to hear the researchers through the headphones, but protected hearing from the MR scanner sounds by attenuating the acoustic noise by approximately 44 db.

Further, participants held a rubber ball with their left hand and were told that the ball was a safety ball and they were to squeeze the safety ball if at any time the scan became uncomfortable, if they felt warmth, tingling, or anything

abnormal, or if they needed to exit the scanner. We checked in with the participants between each scanning sequence to ensure they were feeling OK and were ready to continue. For the baseline MRI and resting-state fMRI, participants were able to see the researchers through a double-sided mirror. For all scans, the researchers were able to view the participants, however given they were laying down in the scanner, the researchers were not be able to see the participant's face.

Statistical Analyses

The analytic plan allowed for preliminary testing of mechanisms in the relationship of acute exercise and executive function. However, the extent of this testing was limited by sample size (MacKinnon et al., 2007) and did not test a moderated-mediation model (as shown in Figure 1). Of importance, testing of the individual relationships proposed, outside of a greater model, is still novel in this field and will inform future research.

Initial Analyses

Participant demographics and Pearson correlations amongst the demographics were examined across the sample as a whole. To test the efficacy of the exercise condition, heart rate and rating of perceived exertion (RPE) were compared across conditions with paired t-tests. Further, differences in reaction time during executive function tasks following exercise and rest were compared with paired t-tests. The difference in reaction time between post-exercise and

post-rest was computed as a relative change score for each test (post-exercise reaction time - post-rest reaction time) for use in further analyses.

BDNF

A preliminary analysis was completed to ensure proper measurement of BDNF with inspection of the standard curve and by computing coefficients of variance (CV). CVs were calculated by dividing the standard deviation of the set of measurements (run in duplicate) by the mean of the set of measurements. Paired-samples t-tests were used to test the difference between pre- and post-condition BDNF. A relative change score was computed comparing change in BDNF following exercise to change in BDNF in response to rest (exercise change-rest change). Linear regressions were used to test relative change in BDNF as a predictor of relative change in behavior.

fMRI

A preliminary analysis was completed to test head motion across conditions. Absolute and relative mean displacement was calculated in FSL prior to performing ICA-AROMA and was reported in millimeters. With ICA-AROMA, the percent of components that were removed was reported. Mean displacement and percent of components removed were compared across conditions with paired-samples t-tests.

Within FSL, post-ICA-AROMA feat directories were entered into FSL for higher-level analyses. A second-level fixed effects model was used to compute comparison of parameter estimate (COPE) images for each subject, during each

trial type, comparing neural activation following each condition. The COPE images from the second-level fixed effects models were used in further third-level mixed effects analyses.

A paired-samples t-test was used to investigate differences in neural activation post-exercise compared to post-rest, while controlling for differences in reaction time. The contrast was two-sided (exercise > rest; rest > exercise). Linear regressions between relative change in neural activation (exercise > rest) and relative change in reaction time (post-exercise – post-rest) were completed for each trial type.

The set shifting task included a 70% switch block (high load) and a 20% switch block (low load). Due to the block design, we were not able to separate switch from repeat trials in the analyses and the hemodynamic responses for both trial types were averaged within each block. Therefore, we were limited to behavioral variables of the highest percent within a block; switch trial reaction time was included for the high load block and repeat trial reaction time was used for the low load block.

To plot findings of neural activation for interpretation, 6mm spheres centered about the peak voxels from significant clusters (i.e. regions where there was a significant change in neural activation result) were created within FSL and used to extract parameter estimates for each participant. Parameter estimates were converted to percent change of BOLD signal within FSL's Featquery tool.

Vagal Tone

A median split was performed on vagal tone (i.e. RMSSD). Independent samples t-tests were used to test the difference between high and low vagal tone on relative change in BDNF, neural activation, and reaction time. All measures of change in neural activation controlled for change in reaction time. Effect sizes for change in reaction time, BDNF, and neural activation were computed and reported as Cohen's *d*.

CHAPTER IV

RESULTS

Demographics

The participants (n=16) were normal to overweight, cognitively normal, older adults (M=72.3 years, SE=1.08). On average participants were highly active (M=592 minutes of MVPA/week, SE=31.1), had an estimated VO_2max of 29.64 ml/kg/min (SE=2.29), and had normal blood pressure (mean arterial pressure M=97.4mmHg, SE=1.96). All demographics are reported in Table 3. Correlations among demographic variables and exercise-related outcomes are reported in Table 4. Total work during the exercise condition was related to minutes of MVPA/week (r=.75, p<.01), VO_2max (r=.67, p<.01), and resting heart rate (r=-.58, p<.05). Additionally, change in BDNF with exercise and relative change in BDNF was associated with rating of perceived exertion during exercise (r=.72, p<.05, r=.79, p<.05, respectively). As expected, average HR (t (15) =12.136, p<.001) and RPE (t (15) =20.38, p<.001) were statistically different between exercise and rest (Figure 5).

Table 3. Participant Demographics. High and low vagal tone groups were split based on a median split of vagal tone. Group comparisons were made between high and low vagal tone groups.

	Overall		Low Vagal Tone		High Vagal Tone				
	n=16 (6 males)		n=7 (4 males)		n=7 (2 males)				
Variable	Mean	SE	Mean	SE	Mean	SE	*t*	df	*p*
Age	72.31	1.08	73.00	1.45	71.29	1.91	0.72	12	.49
BMI	25.06	0.91	26.66	0.96	22.92	1.54	2.07	12	.06
MOCA	27.69	0.25	27.43	0.43	27.86	0.40	-0.73	12	.48
MVPA (minutes)	591.56	124.56	739.29	244.24	505.71	145.05	0.82	12	.43
$VO2max_{n=4}$	29.64	2.29	27.78	3.89	35.07	1.21	-1.53	10	.16
MAP	97.42	1.96	95.81	2.89	95.71	2.46	0.03	12	.98
Medications	1.56	0.40	1.57	0.69	1.29	0.52	0.33	12	.75
Total Work (kcals)	25.16	2.90	29.29	5.08	24.27	3.60	0.81	12	.44
Exercise HRR	50.54%	2.95%	54.90%	1.94%	49.25%	5.24%	1.00	7.61	.35
Exercise RPE	13.26	0.32	13.22	0.65	13.01	0.30	0.30	8.45	.77
Resting HR	64.81	2.95	67.06	4.75	60.66	11.91	0.98	12	.35
$lnRMSSD_{n=15}$	3.22	0.23	2.53	0.27	3.88	0.16	-4.26	12	**.00**
$lnHFpower_{n=5}$	5.12	0.43	3.97	0.58	6.31	0.37	-3.38	12	**.01**
$HF\%_{n=15}$	52.88	8.27	36.15	8.27	52.88	8.34	-1.43	12	.18

BMI= Body Mass Index; MOCA= Montreal Cognitive Assessment; MVPA=moderate to vigorous physical activity minutes per week; VO2max=estimated maximum oxygen uptake; MAP=Mean arterial pressure; Medications: number of prescription medications; Total work is reported in kilocalories (kcals) for the exercise session; Exercise HRR: average heart rate reserve during exercise condition; Exercise RPE: Average rating of perceived exertion during the exercise condition; lnRMSSD=log-transformed root mean square of the successive squared difference; lnHFpower=log transformed high Frequency power; HF% = ratio of high frequency to total power. Sample size is described in second row unless otherwise denoted with a subscript at the variable name.

Table 4. Correlations Across Demographics and Exercise Outcomes.

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. Age	-																
2. BMI	.04	-															
3. MOCA	-.33	.04	-														
4. MVPA (minutes)	.02	-.06	-.20	-													
5. $VO2max_{n=14}$	-.17	**-.73****	-.03	.48	-												
6. MAP	-.07	-.06	.24	-.09	.25	-											
7. Medication	.11	.38	-.13	-.33	-.36	.21	-										
8. Total Work (kcals)	-.15	-.27	-.06	**.75****	**.67****	-.14	-.22	-									
9. Exercise HRR(%)	.41	-.46	-.33	.12	.16	-.34	-.24	.34	-								
10. Exercise RPE	-.08	-.01	-.09	.05	-.22	.17	-.22	-.20	-.17	-							
11. Resting HR	.26	.41	-.16	**-.61***	**-.54***	.28	**.60***	**-.58***	-.04	-.26	-						
12. $lnRMSSD_{n=15}$	-.34	-.25	.33	.03	.37	.10	-.02	-.08	**-.61***	.27	-.48	-					
13. $lnHFpower_{n=15}$	-.23	-.31	.34	.10	.36	.03	-.07	.00	-.43	.31	**-.58***	**.97****	-				
14. $HF\%_{n=15}$	-.02	-.41	.08	-.18	.18	.03	-.07	-.29	-.14	.50	-.27	**.62***	**.68****	-			
15. Exercise $\triangle BDNF_{n=10}$	-.10	-.07	-.25	.15	-.07	-.14	.02	-.16	-.32	**.72***	-.46	.41	.41	.44	-		
16. Rest $\triangle BDNF_{n=9}$	.30	.30	-.46	.41	.10	**-.83****	-.34	.19	-.11	-.05	-.44	.04	.04	-.01	.35	-	
17. Relative $\triangle BDNF_{n=9}$	-.24	-.31	-.10	-.01	-.09	.16	.16	-.26	-.27	**.79***	-.38	.52	.52	.69	**.91****	-.07	-

BMI= Body Mass Index; MOCA= Montreal Cognitive Assessment; MVPA=moderate to vigorous physical activity minutes per week; VO2max=estimated maximum oxygen uptake; MAP=Mean arterial pressure; Medications: number of prescription medications; Total work is reported in kilocalories (kcals) for the exercise session; Exercise HRR: average heart rate reserve during exercise condition; Exercise RPE: Average rating of perceived exertion during the exercise condition; lnRMSSD=log-transformed root mean square of the successive squared difference; lnHFpower=log transformed high Frequency power; HF% = ratio of high frequency to total power. Relative △ BDNF: change in BDNF with exercise – change in BDNF with rest. Sample size: n=16 unless denoted with a subscript. *p<.05; **p<.01.

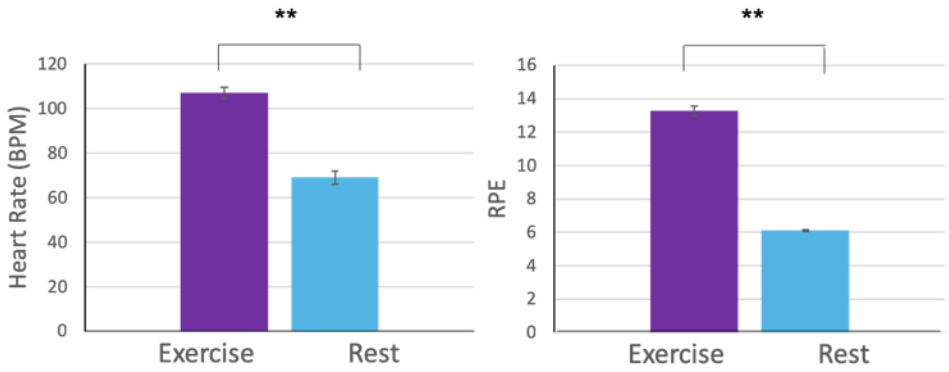

Figure 5. Efficacy of the Exercise Intervention. RPE (rating of perceived exertion) was measured with the Borg's RPE 6-20 scale. **p<.01

Behavioral Outcomes

Out of 16 participants, only 7 were able to reach the predetermined accuracy criterion on the PLUS working memory task on the baseline testing day. Therefore, working memory performance is reported in the initial behavioral analyses, but excluded from further analyses that include BDNF, neural activation, and vagal tone. Paired t-tests revealed no effect of condition on Stroop incongruent trials (p=.62), Stroop neutral trials (p=.58), set shifting high load switch trials (p=.33) and repeat trials (p=.33), set shifting low load switch trials (p=.43) and repeat trials (p=.47), or working memory trials (p=.33). Reaction time measures are presented by trial and condition in Figure 6. Relative change in reaction time was computed for each participant to visually inspect the individual variability of reaction time in response to exercise compared to rest. The individual variability in relative change for each task and trial type is shown in Figure 7.

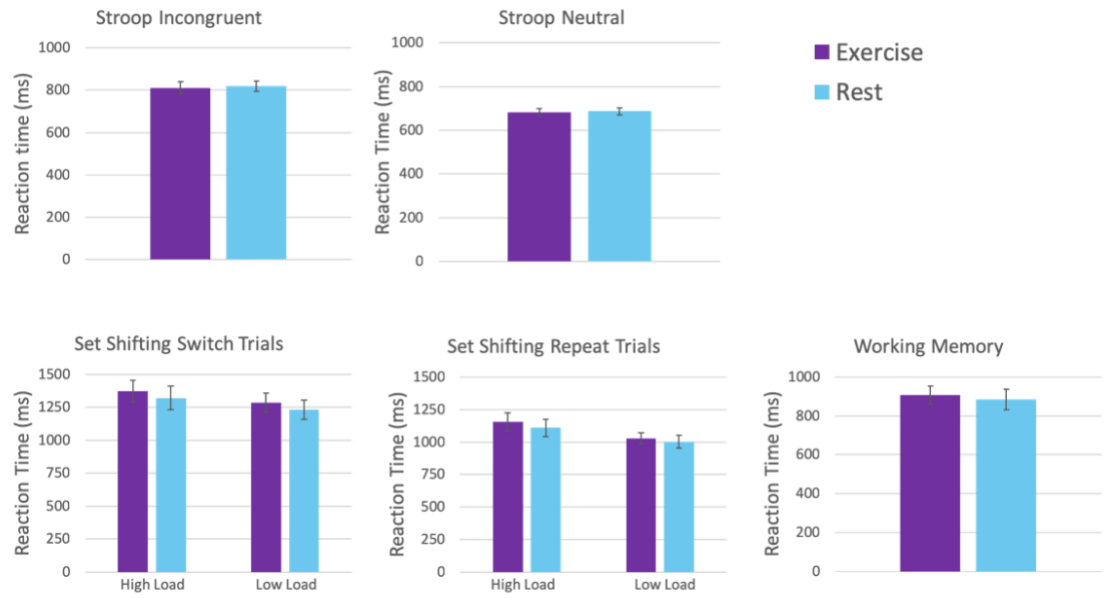

Figure 6. Reaction Time Across Each Task and Trial Type by Condition.

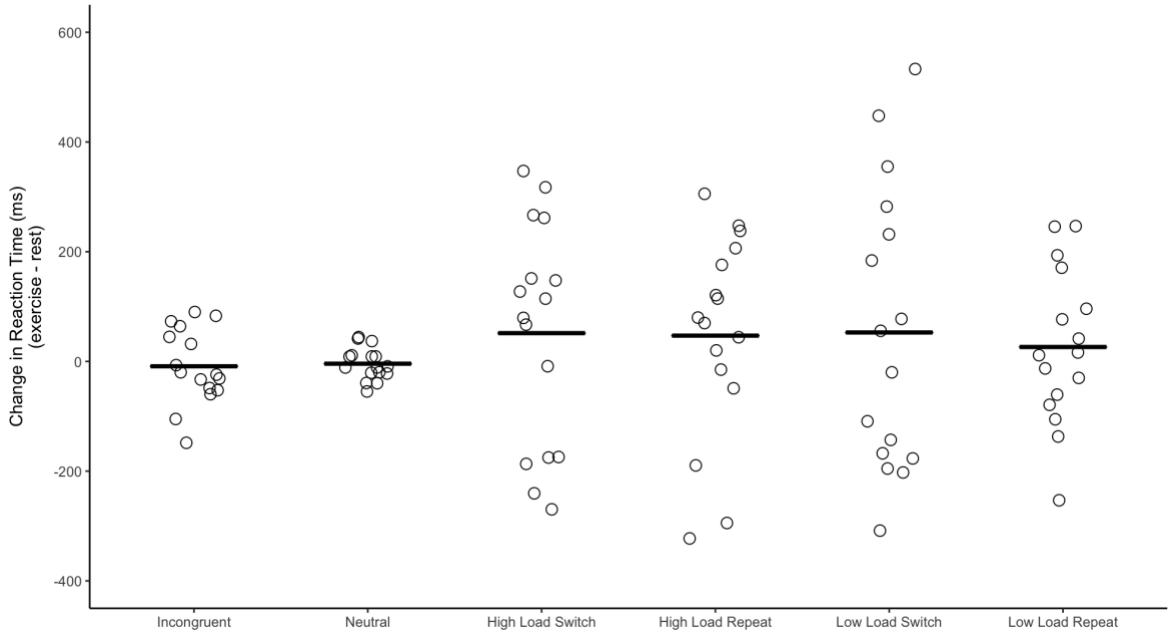

Figure 7. Individual Variability in Relative Change in Reaction Time.

Brain-Derived Neurotrophic Factor

Preliminary Analyses

Nine participants had BDNF measures at all time points and one participant had BDNF only for the exercise condition. The standard curve for the BDNF ELISA is presented in Figure 8. The CVs ranged from 0.06% to 14.5%, with five CVs between 10-14.5%.

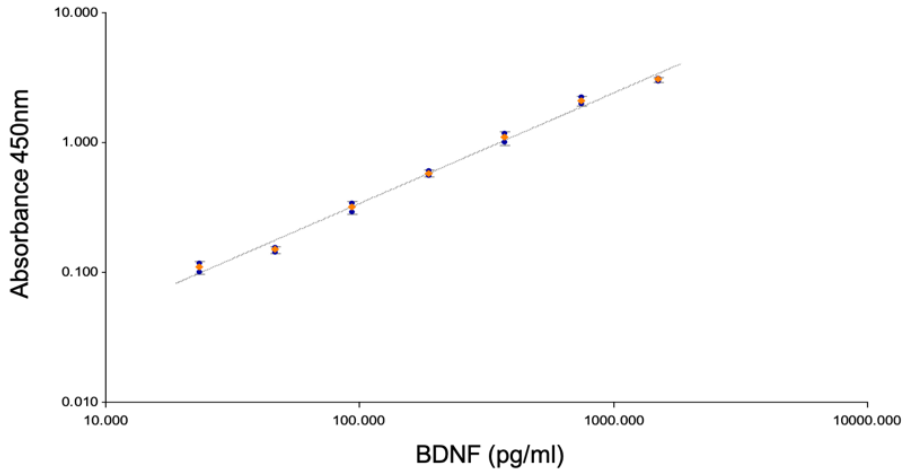

Figure 8. BDNF Standard Curve. The standard curve for the Aviscera Bioscience Brain Derived Neurotrophic Factor (BDNF) enzyme linked immunosorbent assay.

BDNF Outcomes

For the rest condition, average BDNF was 34.89 ± 2.24 ng/ml at pre and 33.02 ± 2.32 ng/ml at post. For the exercise condition, average BDNF was 31.75 ± 2.33 ng/ml at pre and 29.72 ± 1.71 at post. Results of the paired t-tests comparing pre-to post-condition were not significant for exercise (t (9) =1.08, p=.31) or rest (t (8) =2.23, p=.06). Relative change in BDNF (change with

exercise – change with rest) was computed to visually inspect individual variability in the response of BDNF and is shown in Figure 9.

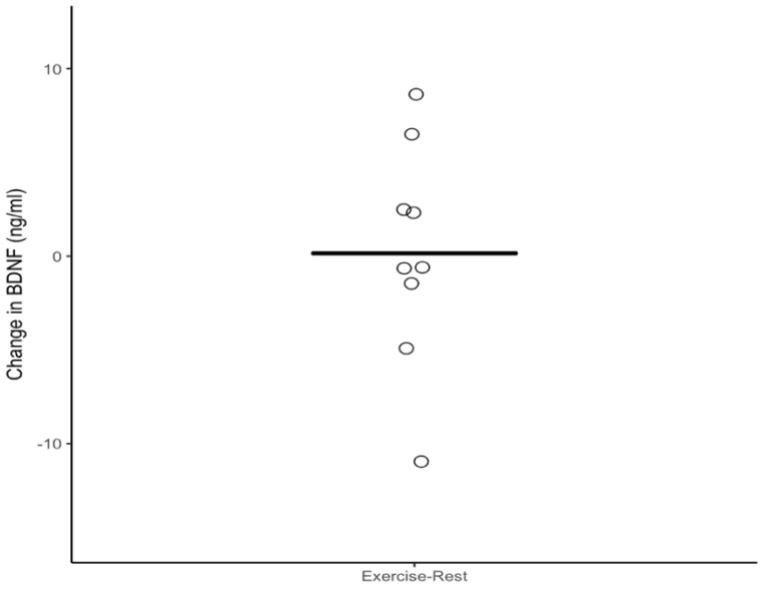

Figure 9. Individual Variability in Relative Change in BDNF. Relative change computed as change in BDNF with exercise minus change in BDNF with rest.

Relative change in BDNF (exercise change-rest change) did not predict relative change in incongruent (β =-.60, *p*=.09, r^2=.36) or neutral (β =-.44, *p*=.24, r^2=.07) reaction time. Relative change in BDNF was a significant predictor for high load switch trials (β =-.76, *p*=.02, r^2=.58), high load repeat trials (β =-83, *p*=.01, r^2=.69), low load switch trials (β=-.82, *p*=.01, r^2=.67), and low load repeat trials (β =-77, *p*=.02, r^2=.59). A visual depiction of the relationship between change in BDNF and all of the set switching variables is presented in Figure 10.

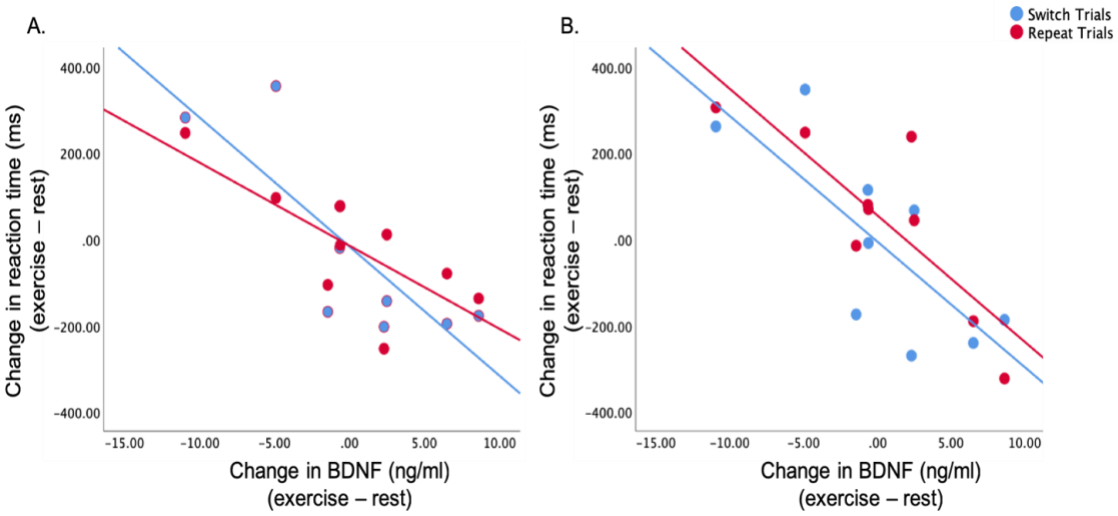

Figure 10. Change in BDNF and Change in Set Shifting. A) Significant relationships during low load trial blocks. B) Significant relationships during high load trial blocks.

Neural Activation

Preliminary Analyses

Head motion, measured as absolute and relative mean displacement (mm), is reported in Table 5 for each condition and trial type. ICA-AROMA resulted in a classification of 69.18% of the total components as motion and removed them from analyses. Around 70% of component removal is expected with automated noise removal (Griffanti et al., 2017, 2014; Rummel et al., 2013). Percent of components removed for each condition and trial type are reported in Table 5. Head motion parameters and percent of components removed did not vary across condition for absolute or relative parameters ($p>.05$), however during the Stroop test, the percent of components removed was larger following rest compared to exercise (t (15) =-1.80; p=.009).

Table 5. Head Motion during fMRI. Pre-ICA-AROMA motion and the results of ICA-AROMA (percent of components removed) are reported.

	Stroop			Set Shifting		
	Exercise	Rest	*p*	Exercise	Rest	*p*
Absolute motion (mm)	0.45 (.05)	0.51 (.09)	.53	0.40 (.06)	0.64 (.12)	.06
Relative motion (mm)	0.07 (.01)	0.09 (.01)	.09	0.08 (.01)	0.09 (.01)	.39
Percent of Components Removed	68.50% (.02)	75.34% (.03)	.00	69.10% (.02)	71.80% (.03)	.34

Effect of Condition on Neural Activation

During the incongruent trials, there was greater activation in the left cerebellum VIIIa and reduced activation in the right Crus I with exercise compared to rest (Figure 11).

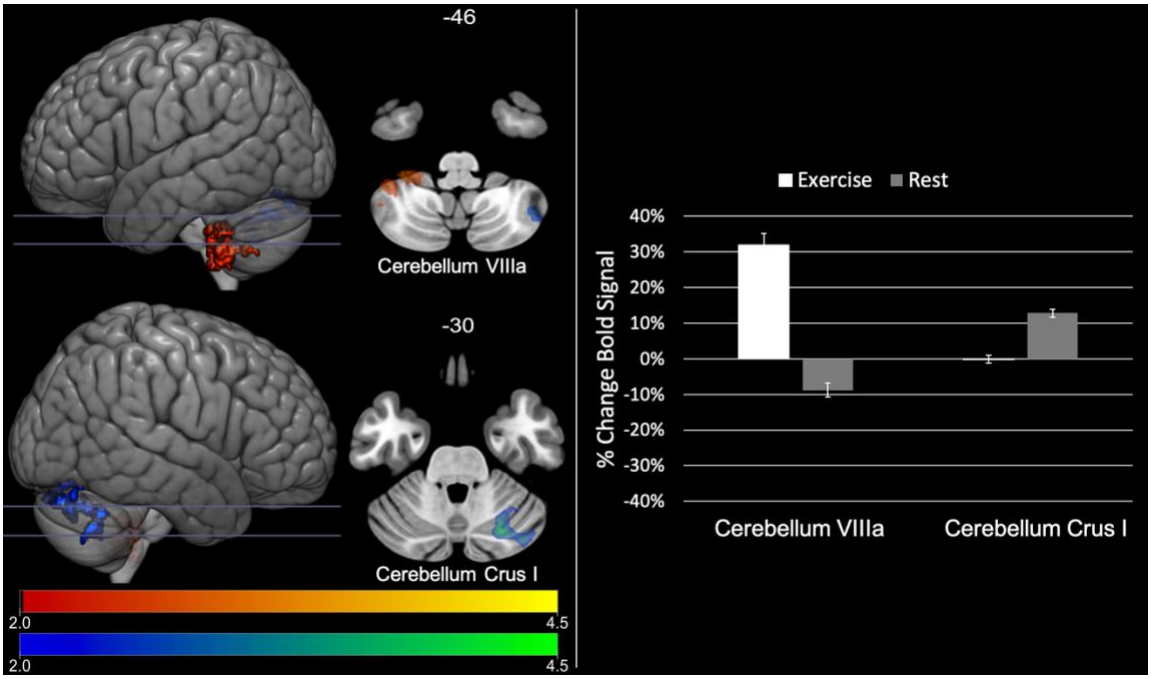

Figure 11. Activation Differences During Incongruent Trial Blocks. Regions where exercise elicited greater activation compared to rest is shown in red. Regions where exercise elicited reduced activation compared to rest is shown in blue. See Table 6 for test statistics and coordinates.

During the neutral trials, there was greater activation of the right frontopolar area (BA 10) and reduced activation in the right Crus I, supramarginal gyrus (BA 40), and superior frontal gyrus (BA 6) with exercise compared to rest (Figure 12).

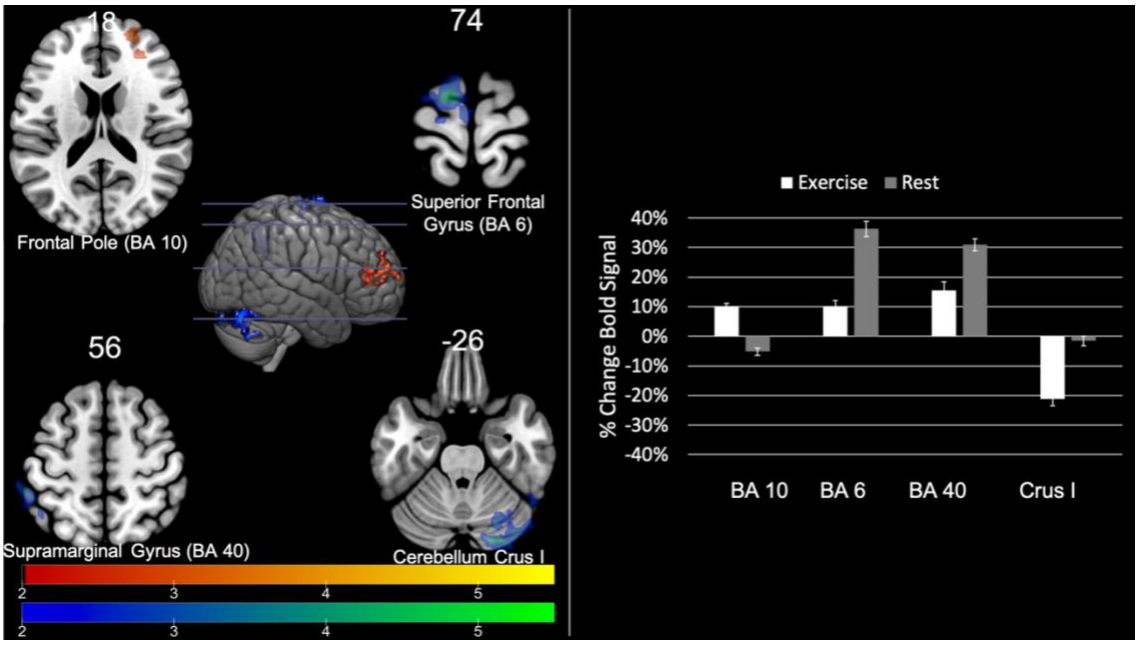

Figure 12. Activation Differences During Neutral Trial Blocks. Regions where exercise elicited greater activation compared to rest is shown in red. Regions where exercise elicited reduced activation compared to rest is shown in blue. See Table 6 for test statistics and coordinates.

During the high load trials, there was reduced activation in the subcallosal cortex (BA 25) and the middle frontal gyrus (BA 6) with exercise compared to rest (Figure 11). During the low load trials, there was reduced activation in the superior frontal gyrus (BA 8) with exercise compared to rest (Figure 13).

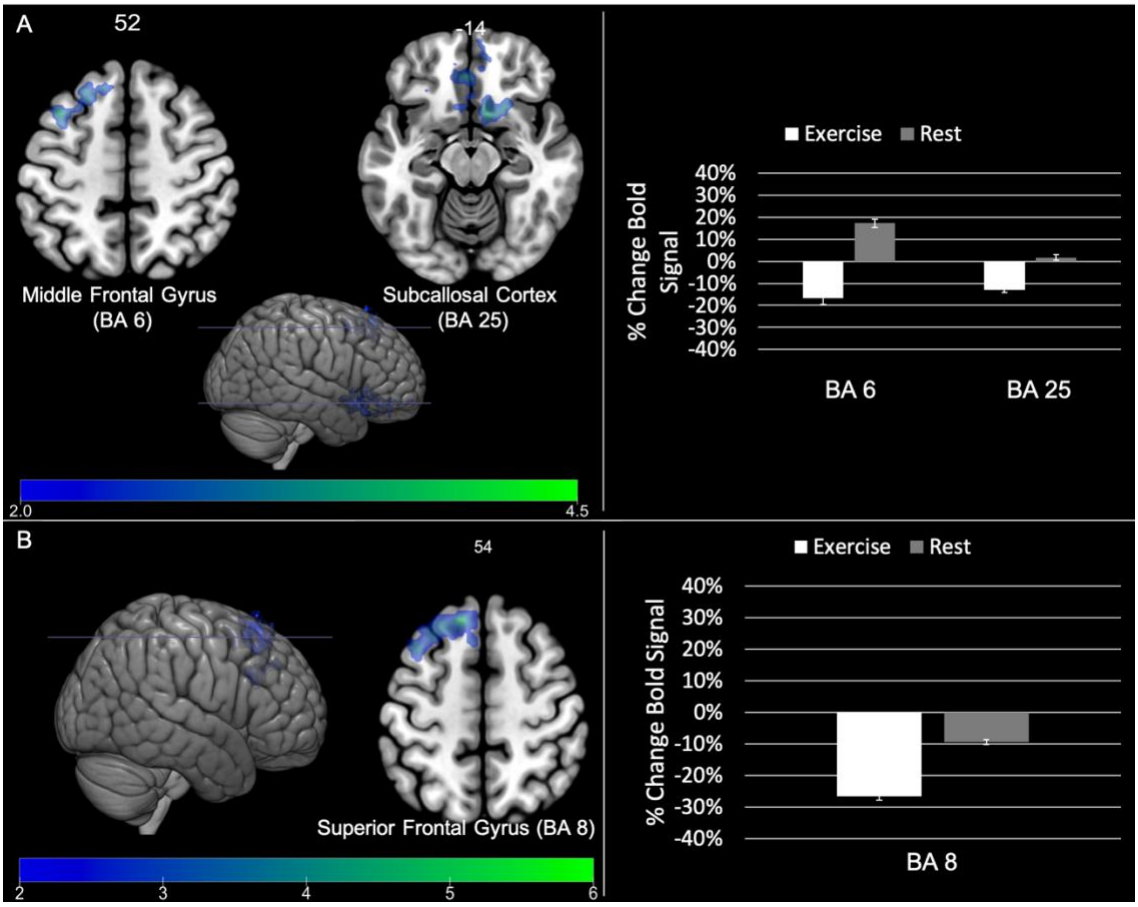

Figure 13. Activation Differences During Set Shifting Trial Blocks. High load trial block differences shown in A, Low load trial block differences shown in B. Regions where exercise elicited reduced activation compared to rest is shown in blue. See Table 6 for test statistics and coordinates.

Neural Activation and Executive Function

Relationships between change in neural activation and change in reaction time are presented in Figure 14. Change in reaction time with exercise during the incongruent trials was not related to change in neural activation. During the neutral trials, change in reaction time was associated with change in neural

activation such that greater activation in the postcentral gyrus (BA 1) with exercise was associated with reduced reaction time.

During the high load blocks, change in switch trial reaction time was associated with neural activation such that reduced activation in the inferior frontal gyrus (BA 44) and greater activation in the supramarginal gyrus (BA 40) with exercise was associated with reduced reaction time. During the low load blocks, change in repeat trial reaction time was associated with neural activation such that reduced activation in the frontopolar area (BA 8, BA 46, BA 10) was associated with reduced reaction time.

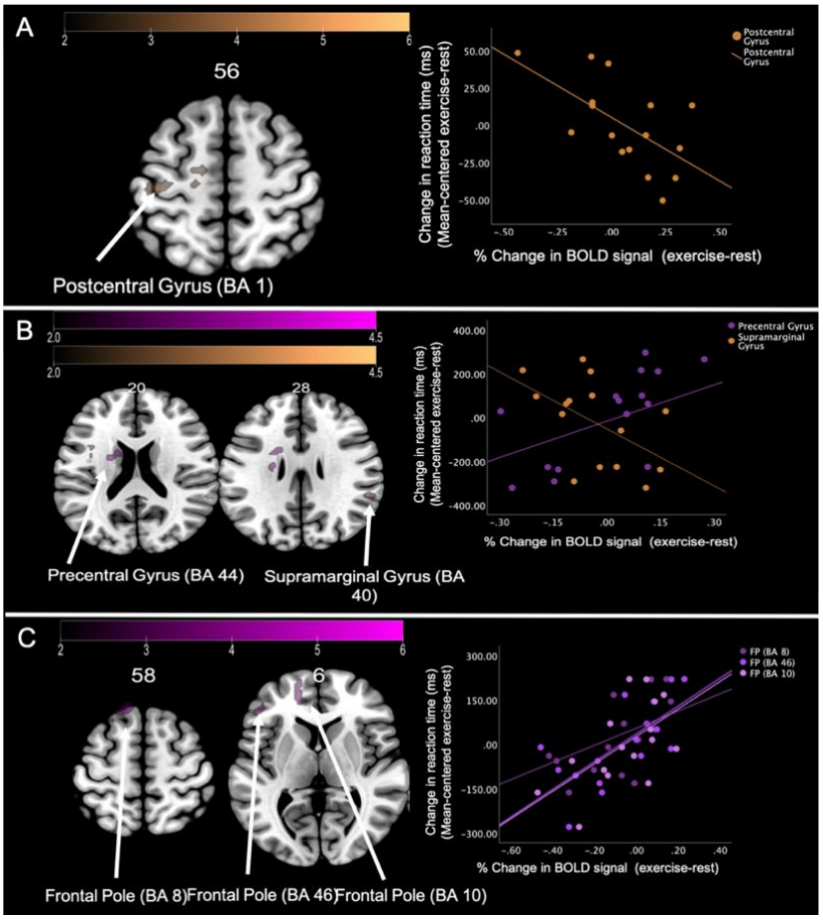

Figure 14. Change in Activation and Change in Behavior with Exercise. A) Significant relationships during the neutral trial blocks. B) Significant relationships during the high load trial blocks. C) Significant relationships during the low load trial blocks. Regions identified with a positive relationship are shown in purple and negative relationship shown in copper. See Table 6 for test statistics and coordinates.

Table 6. Local Maxima of Significant Clusters from fMRI Analyses. Regions shown were identified when comparing exercise and rest conditions or change in activation with change in behavioral outcome.

Trial Block	Contrast	Region	BA	H	Voxels	x	y	z	z-max	*p*
Effect of Condition										
Incongruent	E > R	Cerebellum VIIIa	-	L	495	-30	-34	-46	3.74	.01
	R > E	Cerebellum Crus I	-	R	516	26	-70	-30	4.31	.00
Neutral	E > R	Frontal Pole	10	R	384	24	56	18	3.54	.03
	R > E	Cerebellum Crus I	-	R	666	28	-84	-26	5.1	.00
		Supramarginal Gyrus	40	L	525	-54	-40	56	3.93	.00
		Superior Frontal Gyrus	10	L	466	-14	-6	74	4.8	.01
High Load	R > E	Subcallosal Cingulate	25	R	886	10	12	-14	3.61	.00
		Middle Frontal Gyrus	6	L	565	-38	16	52	3.63	.00
Low Load	R > E	Superior Frontal Gyrus	8	L	1115	-12	32	54	5.62	.00
Relationships with Behavior										
Neutral	Negative	Postcentral Gyrus	1	L	748	-44	-22	56	4.33	.00
High Load	Positive	Precentral Gyrus	44	L	551	-40	10	20	4.09	.00
		Supramarginal Gyrus	40	R	414	58	-42	28	3.86	.02
Low Load	Positive	Frontal Pole	8	L	1045	-12	38	58	4.86	.00
		Frontal Pole	46	L	466	-46	38	6	4.38	.01
		Frontal Pole	10	L	364	-16	60	8	3.63	.03

BA: Brodmann area; H: Hemisphere; peak coordinates shown as x, y, z mm. Contrasts R > E: rest > exercise; E > R: exercise over rest

Vagal Tone

Fifteen participants had measures of vagal tone and were included in analyses. The participant whose measure of vagal tone was precisely at the median was dropped from analyses and groups were identified as high (n=7) and low (n=7) vagal tone. The differential effects on behavioral, BDNF, and neural activation are presented below and shown in Figure 15. The results in Figure 14 are separated by level of vagal tone (high/low) and visually depict the responses to exercise on each dependent variable by subject. A focus of this study has been to account for the individual variability of the effects of acute exercise.

Although plotting means of groups can be beneficial, recognizing that there positive and negative responders, as well as non-responders, will help to provide meaning to moderators and/or highlight the need for future investigations.

Vagal Tone and Behavioral Outcomes

Compared to those with low vagal tone, participants with high vagal tone had significantly lower reaction time following exercise during incongruent (t (12) =2.33, p=.04, d=-1.25) and high load repeat trials (t (12) =2.47, p=.03, d=-1.32). Although not significant, compared to low vagal tone, those with high vagal tone had reduced reaction time in neutral trials (d=-.31), high load switch trials (d=-.55), low load switch trials (d=-.31), and low load repeat trials (d=-.58) with exercise, relative to rest.

Vagal Tone and BDNF

Although not significant, compared to low vagal tone, those with high vagal tone had increased BDNF (d=.80) in response to exercise, relative to during rest.

Vagal Tone and Neural Activation

Out of the regions that differed between exercise and rest conditions, the cerebellum VIIIa during the incongruent trials of the Stroop, the superior frontal gyrus during the neutral trials of the Stroop, and the subcallosal cortex during the high load blocks of the set shifting test further differed between high and low vagal tone. The cerebellum VIIIa had significantly greater activation following exercise compared to rest, however this effect was greater for those with high

vagal tone compared to lower vagal tone (*t* (12) =-2.23, *p*=.05, *d*=1.19). The superior frontal gyrus had reduced activation following exercise compared to rest, however that reduction was greater in those with low vagal tone compared to higher vagal tone (*t* (12) =-2.59, *p*=.03, *d*=1.38). Finally, the subcallosal cortex had reduced activation following exercise compared to rest, however that reduction was greater in those with low vagal tone compared to higher vagal tone (*t* (12) =-2.24, *p*=.05, *d*=1.20).

Figure 15. Outcomes Stratified by High and Low Vagal Tone. Differential effects on executive function (A), BDNF (B), and neural activation (C) by level of vagal tone. Low vagal tone is shaded in light blue on the left and high vagal tone is shaded in light red on the right. Data is presented at the subject-level and the numbers correspond to the same subject across all measures.

CHAPTER V

DISCUSSION

The aim of this study was to investigate potential mechanisms involved in the acute exercise and executive function relationship in older adults. Using a within-subjects counterbalanced design, we asked older adults to complete 30 minutes of moderate-intensity exercise and 30 minutes of rest on separate days. We assessed the differences in responses to exercise versus rest on measures of executive function, BDNF, and neural activation.

Behavioral Outcomes

There were no differences in executive function following exercise compared to following rest. In a recent meta-analyses, it has consistently been found that acute exercise benefits executive function (Chang et al., 2012; Lambourne & Tomporowski, 2010; Ludyga et al., 2016; McMorris & Hale, 2012). Further, when age of participants is considered as a moderator, there are larger benefits for older adults (Chang et al., 2012; Ludyga et al., 2016). Our findings here were unexpected, as they do not corroborate the findings of multiple meta-analyses. A potential explanation for our lack of significance may be due to the delay between exercise and cognitive testing and the characteristics of the exercise itself.

Though we made great attempts to minimize the delay between cessation of exercise and onset of testing (e.g. restroom breaks prior to exercise, efficient blood draws, pre-condition MRI/fMRI to habituate to the scanning environment and learn safety information), the average time delay was approximately 15 minutes. The benefits of exercise on cognition may be affected by the time-delay from exercise to start of testing. A post-exercise cut off for the positive effects of acute exercise on cognition has been suggested by meta-analytic findings at 15 minutes (Chang et al., 2012; Lambourne & Tomporowski, 2010), however individual studies have suggested this benefit lasts well beyond 15 minutes, suggesting positive effects for about 50 minutes (Joyce, Graydon, McMorris, & Davranche, 2009).

In addition, the duration of exercise may have affected the results. Chang et al. (2012) found that 11-20 minutes of exercise is the ideal duration of exercise to benefit cognitive performance, with a negative effect of 1-10 minutes and a smaller magnitude positive effect with greater than 20 minutes. Though executive function was a major component of this study, we also included BDNF, as a suggested mediator, for which there is evidence that at least 30 minutes is needed to have a positive effect (Dinoff et al., 2017). The duration was set based on the BDNF meta-analysis and prescribed for 30 minutes, with an understanding that the magnitude of effect for executive function may be smaller, but it would still be beneficial. This decision may have influenced the effects on executive function and is further discussed below in the BDNF section.

The delay between exercise and cognitive testing, as well as the duration of the exercise, may have attributed to our lack of significant findings here, however the benefits of acute exercise on cognition have consistently been of a small magnitude, with numerous factors affecting the magnitude of the relationship. Although we expected benefits to executive function, we report that on average in our small sample of older adults there were no benefits, however this does not suggest that no one in our study experienced benefits (Figure 7). To further investigate individual variability in the response to exercise, we will turn the discussion to our proposed mechanistic variables of BDNF, neural activation, and vagal tone.

Brain-Derived Neurotrophic Factor

Exercise did not have an overall effect on BDNF. The pre-condition BDNF reported here was slightly higher than previous studies reporting raw BDNF values in older adults. Pre-rest and pre-exercise values for this sample were about 35 and 32 ng/ml, respectively, with previous studies showing baseline average serum BDNF of about 20-25 ng/ml for similarly-aged older adults (Erickson et al., 2010; Laske et al., 2010; Hakansson et al. 2017). However, Erickson et al. (2010) plotted resting serum BDNF levels for 142 older adults, which ranged from about 5 ng/ml to about 45 ng/ml. In the results of the ELISA, the CVs were low and the measured values are within reason for this age sample based on previous literature. Therefore, we do not believe the variability in response to rest or exercise was due to measurement error.

The duration of exercise chosen for this study was based on a recent meta-analysis reporting that exercise sessions lasting at least 30 minutes had greater positive effects on BDNF as compared to sessions less than 30 minutes (Dinoff et al., 2017). Interestingly, following 30 minutes of exercise, our sample of older adults did not experience a significant change in BDNF. Given the variability in the response of BDNF to exercise, relative to the rest control condition, in our older adult sample (Figure 4), the duration cutoff as suggested by Dinoff et al. (2017) may not have been the ideal choice for this sample (M=72.3 years) which was substantially older than the average age of studies included in the meta-analysis (M=27.9 years). Future acute exercise studies with older adults should consider including BDNF to advance our understanding of the ideal exercise characteristics to positively affect BDNF.

Our hypothesis stated that change in BDNF would be associated with change in executive function following exercise. Though change in BDNF with exercise, relative to change with rest, varied across individuals, it was predictive of change in reaction time on the category switching test, a measure of cognitive flexibility, and accounted for over 58% of the variance. BDNF was not related to performance on the Stroop test, a measure of inhibitory control. This is consistent with a recent study showing change in BDNF in response to exercise is associated with change in cognitive flexibility, but not change in inhibitory control in young adults (Hwang et al., 2016). The latter finding, a lack of relationship between change in BDNF and change in inhibitory control, has additional support

(Ferris et al., 2007). Given the consistency in the relationship between BDNF and cognitive flexibility, but not inhibitory control, in old and young adults, there may be a specificity of the downstream effects of BDNF on certain cognitive domains. Cognitive flexibility tasks require participants to learn a set of rules, remember the rules, and respond appropriately based on the rules. Therefore, although switching back and forth between the rules may be a cognitive flexibility/executive function-based task, the ability to remember the rules has a necessary level of learning and memory (Braem & Egner, 2018). The Stroop test requires memory for simple rules (e.g. “respond to the color of the ink” or “read the word”), but there is a lack of complexity as compared to cognitive flexibility paradigms.

The importance of BDNF in learning and memory has been widely investigated, recognized, and reviewed (Bekinschtein et al., 2007; Cunha, 2010). However, the relationship between change in BDNF in response to exercise with change in learning and memory is controversial, with some showing positive findings (Lee et al., 2014; Skriver et al., 2014; Winter et al., 2007) and others finding no relationship (Etnier et al., 2016; Hötting, Schickert, Kaiser, Röder, & Schmidt-Kassow, 2016). Interestingly, the studies that found a positive relationship between BDNF and behavior following exercise used a visual task; whereas, the studies finding no relationship utilized an auditory task. Visual learning and memory recall are superior to auditory recall (Cohen, Horowitz, & Wolfe, 2010) and perhaps the upregulation of BDNF with exercise has a specific

role in visual sensory integration with the hippocampus. Though theoretical, the specificity to visual stimuli may have been overlooked when translating the animal literature to human literature, given that animal studies are reliant on visually-dependent tasks (Quillfeldt, 2015), and this may be an important factor to consider in the future.

Although the relationship between the response of BDNF to exercise with learning and memory may differ by stimulus type, cognitive flexibility tasks used here and in a similar study (Hwang et al., 2016) presented visual stimuli and found a relationship between change in BDNF in response to exercise associated with change in behavior. Perhaps the combination of use of visual stimuli, a reliance on learning and memory for the task rules, and acute exercise, created a scenario for which BDNF-dependent benefits on cognitive flexibility were achieved.

Neural Activation

With use of fMRI, acute exercise has been shown to affect neural activation in frontal (Chen et al., 2016; Li et al., 2014; Metcalfe et al., 2015), medial temporal lobe (Chen et al., 2016; Metcalfe et al., 2015), and cerebellar (Chen et al., 2016) regions. Though these effects are important in initially recognizing that exercise can affect neural activation, the downstream effects on cognition are not consistent and literature with older adults is lacking, with this study being the first to investigate in older adults with task-based fMRI. Here we explored the effects of acute exercise on neural activation in older adults with

whole-brain analyses, while controlling for behavioral performance. To date, the published studies examining brain and behavior using fMRI have reported differences by condition (i.e., exercise vs control) and/or trial type, however we were also interested in the relation between change in neural activation and change in behavior by condition. Though the differences in activation are informative in terms of effects of exercise on the brain, linking changes by condition with change in performance was a strength of this study to better understand changes in neural activation as a mechanism.

Effect of Condition

Inhibitory control.

The incongruent and neutral trial blocks of the Stroop tested inhibitory control and processing speed, respectively. During inhibitory control trial blocks, there was a significant effect of condition on the cerebellum VIIIa and the crus I. After plotting the parameter estimates, it became evident that with exercise there was a positive effect on neural activation in the cerebellum VIIIa, while with rest there was minimal change. The cerebellum VIIIa is involved with sensorimotor processing (Stoodley, Valera, & Schmahmann, 2010) and is functionally connected to the prefrontal cortex (Krienen & Buckner, 2009), which is involved with executive processing. An increase in activation with exercise may suggest compensation for inhibitory control through increasing the resources available for processing downstream in the prefrontal cortex.

The effect of condition on the Crus I during inhibitory control trial blocks showed positive activation with rest and no change with exercise. Interestingly, in the processing speed blocks, this finding was reversed such that after rest there was no change and with exercise there was substantial deactivation. The Crus I is implicated in the default mode network (Krienen & Buckner, 2009), which is associated with task-related deactivation (Anticevic et al., 2012; Raichle et al., 2000). The extent of task-induced deactivation is usually reduced in older adults (Andrews-Hanna et al., 2007; Grady et al., 2010; Persson, Lustig, Nelson, & Reuter-Lorenz, 2007), which is suggested to be compensatory in order to maintain behavioral performance (Grady et al., 2010). In the more challenging trial blocks (i.e. inhibitory control), neither exercise nor rest elicited deactivation, however there was minimal change with exercise and positive activation with rest. In the less challenging trial blocks (i.e. processing speed), exercise allowed for a greater deactivation. The findings here suggest that exercise benefitted the default mode network processes and improved efficiency during trials.

Additionally, during the processing speed trial blocks, exercise yielded positive activations in frontal regions, however in the supramarginal gyrus and the superior frontal gyrus the positive activation was to a lesser extent than following rest. Given the lack of differences in behavior, this suggests exercise improved neural efficiency with reduced resources needed for the same behavioral outcome.

Cognitive flexibility.

The high load and low load trial blocks during the set shifting task tested shifting, as a measure of cognitive flexibility, and processing speed, respectively. Though there were switch and repeat trials in both conditions, the high load blocks were heavily weighted towards shifting and low load heavily weighted towards processing speed. During the high load shifting trial blocks, there was an effect of condition on the subcallosal cortex and the middle frontal gyrus. Following exercise, there was a deactivation of the subcallosal cortex and with rest there was positive activation. This region is sometimes combined with the anterior cingulate cortex due to BA classifications, however, others suggest that it should be maintained separately (Hamani et al., 2011) and thus we will discuss it as a separate region. The subcallosal cortex has positive activity with depressive thoughts (Hamani et al., 2011) and demonstrates a deactivation following the administration of anti-depressive medication (e.g. selective serotonin-reuptake inhibitors; (Mayberg et al., 2002). Further, a deactivation has been recognized as a part of the placebo effect and is associated with reduction in depressive symptoms (Mayberg et al., 2002). Exercise has been implicated in improvements in mood (Bartholomew, Morrison, & Ciccolo, 2005; Crush, Frith, & Loprinzi, 2018; Reed & Ones, 2006; Yeung, 1996) which has been suggested to lead to improved cognitive performance (Basso & Suzuki, 2017). Perhaps acute exercise improved mood, leading to a reduction in activation of the subcallosal cortex during a particularly taxing cognitive test.

In addition, during high load shifting trial blocks, the left middle frontal gyrus exhibited a deactivation following exercise, whereas there was minimal change with rest. The left middle frontal gyrus is implicated in the default mode network and following the same line of reasoning that we found with the effect of exercise on other default mode regions, exercise appears to be improving the task-induced deactivation, while with rest you see no change or positive activation suggesting less efficiency and potentially compensation for age-related declines.

During low load shifting trial blocks, there was deactivation in the superior frontal gyrus following both exercise and rest, however the extent of the deactivation was greater following exercise. In contrast to our other findings, this was not expected as the superior frontal gyrus (BA 8) is highly involved with cognitive control (Niendam et al., 2012), and positive activation would be expected similar to what was seen during the processing speed trials of the Stroop test. However, although the low load shifting blocks were 80% repeat trials, they included 20% shifting trials. Perhaps this effect of condition was specific to the shifting trials, but the inferences that can be made here are limited given the inconsistencies between the current finding and the extant literature.

Change in Neural Activation with Change in Behavior

Inhibitory control.

There were no apparent relationships between change in neural activation during the inhibitory control trial blocks and change in behavior, however there

was for the processing speed trials. During processing speed trial blocks, greater activation in the postcentral gyrus following exercise, relative to rest, was associated with improved reaction time. The postcentral gyrus is a sensory region of the brain that is associated with attention (Balslev, Odoj, & Karnath, 2013) and perhaps with greater activation, there was a greater level of attention and faster processing of sensory information which led to improved reaction time.

Cognitive flexibility.

During high load shifting trial blocks, reduced activation in a default mode network region (inferior frontal gyrus) following exercise, compared to rest, was associated with improved reaction time for switching trials. Further, within a cognitive control region (supramarginal gyrus) greater activation following exercise, compared to rest, was associated with improved reaction time for switching trials. These findings are consistent with the hypothesis that exercise supports the default mode network and the cognitive control network and responding to exercise in this way leads to improved performance.

In the low load shifting trial blocks, reduced activation following exercise in three cognitive control regions (BA 8, DLPFC, and BA10), compared to rest, was associated with improved reaction time for repeat trials. These regions are associated with cognitive control (Niendam et al., 2012), thus a reduction in activation with improved behavior suggests efficiency during trials that are heavily weighted towards processing speed, rather than cognitive control.

Altogether, these findings suggest an important effect of acute exercise on neural activation during executive function tasks. It appears that exercise bolsters the default mode network and improves the efficiency of the cognitive control network in older adults. Both of these networks are known to experience substantial changes in task-related activation, as well as functional connectivity, which are associated with age-related declines in performance. Though there were no differences in behavior between the conditions, these findings suggest that exercise affects neural activation and those that experience certain changes in neural activation have improved performance in response to exercise.

Vagal Tone

Our discussion thus far has been focused on relationships between change in potential mediators affecting executive function in response to exercise. In addition to recognizing mediators, identifying moderators that may affect the magnitude and/or direction of the response to exercise was of importance in this study. Though the cognitive reserve theory provides a framework for individual variability in aging, accounting for cognitive reserves is complicated. Greater cognitive reserves are associated with resiliency against aging and pathological declines, and those with reduced reserves may have a greater window for improvement in response to interventions, such as exercise (Stern, 2009). Some have attempted to quantify cognitive reserves with the Cognitive Reserve Index (Nucci, Mapelli, & Mondini, 2012), however quantification of cognitive reserves with a measure that encompasses factors

that cannot be changed (e.g. highest education achieved, occupation) disqualifies the measure as a target for interventions. Further, it is known that interactions between previous and current lifestyle choices and other factors, such as genetics, can influence the level of cognitive reserves (Stern, 2009). Here we proposed vagal tone as a meaningful measure that is associated with multiple individual factors (e.g. health status, age, sleep, fitness, genetics) and, of importance, is malleable and could potentially serve as a target for future interventions. Low vagal tone is associated with poor health and cognitive outcomes, and thus may reflect lower cognitive reserves.

The cognitive reserve theory posits that those with lower cognitive reserves have a greater window for improvement; thus, it was hypothesized those with lower vagal tone would have a greater benefit from exercise. Vagal tone was measured by RMSSD and split into high and low vagal tone groups with a median split. The overall average of log-transformed RMSSD in this sample was 3.22, with a range of 1.22-4.52. Normative data for log-transformed RMSSD in healthy adults has been reported as an average of 3.49 and a range of 3.26-3.41 (Nunan, Sandercock, & Brodie, 2010). Although the range in this study was much larger than the normative range, when compared to individual studies reporting log-transformed RMSSD in older adults, the values we report are within reason (Albinet et al., 2016, 2010; Almeida-Santos et al., 2016).

Behavioral Outcomes and BDNF

Interestingly, albeit non-significant, those with higher vagal tone had consistently better outcomes in response to exercise with executive function and BDNF compared to those with lower vagal tone. Though this was the opposite direction as hypothesized, the benefits achieved with acute exercise for those with higher vagal tone in this study may suggest the system was primed to respond. Vagal tone is a holistic measure of the system and those with higher vagal tone have higher levels of BDNF (Yang et al., 2010) and executive function (Gillie & Thayer, 2014; Hansen et al., 2003; Thayer et al., 2012). BDNF expression and signaling through its high affinity receptor, TrkB, are associated with benefits to cognitive performance (Croll, Ip, Lindsay, & Wiegand, 1998; Ding, Ying, & Gómez-Pinilla, 2011). Given that those with higher vagal tone have higher BDNF expression, they may experience greater acute benefits in behavioral outcomes and BDNF, with more effective downstream signaling. In support of this explanation, we found that those with higher vagal tone experienced greater improvements from rest to exercise in executive function, which varied by measure from a small to large magnitude of effect. Further, the change in BDNF from rest to exercise was higher for those with higher vagal tone with a large magnitude of effect.

If this were to be extended into a chronic exercise intervention, the cognitive reserve theory, as well as our initial hypothesis, would support that those with lower vagal tone would experience greater benefits than those with

higher vagal tone due to having a greater window for improvement. In fact, chronic exercise is associated with increased vagal tone (Albinet et al., 2016, 2010) and BDNF expression (Leckie et al., 2014), both of which are associated with downstream benefits on executive function (Albinet et al., 2016; Leckie et al., 2014). The benefits of chronic exercise on BDNF and executive function have been shown to be greater in those in a more declined state (i.e. oldest-old adults; Leckie et al., 2014), who would also be expected to have reduced vagal tone. Of importance, following an exercise intervention the response to acute exercise may vary, given the differential "priming" of the system. Future studies should consider including a response to acute exercise measure pre and post chronic intervention to provide a measure of physiologic adaptations to the system in response to exercise. It may be expected that those with greater benefits from chronic exercise also experience greater benefits from acute exercise.

Neural Activation

Vagal tone influenced activation in three regions that were affected by exercise, the cerebellum VIIIa, superior frontal gyrus, and subcallosal cortex. With exercise, there was increased activation in the cerebellum VIIIa, relative to rest, and those with higher vagal tone increased activation to a greater magnitude than those with lower vagal tone. Earlier it was suggested that this effect of condition may be compensatory as the cerebellum VIIIa is highly connected to the prefrontal cortex and activation here may be indicative of increasing available resources for higher cognitive regions (e.g. prefrontal

cortex). Higher vagal tone was associated with improved executive function with exercise and this greater magnitude of activation may help to explain that relationship.

Interestingly, lower vagal tone appeared to benefit other regions affected by exercise. The superior frontal gyrus had reduced positive activation following exercise, which was suggested as neural efficiency, but those with lower vagal tone had a reduction in activation following exercise compared to those with higher vagal tone. Consistent with the cognitive reserve theory, this suggests that those with lower vagal tone had greater room for improvement. These findings indicate that the difference between rest and exercise conditions was greater for those with lower vagal tone, however this could mean that those with higher vagal tone had more efficient activation with rest and exercise, thus changing to a lesser extent. This is consistent with our hypotheses that those with lower vagal tone would experience greater changes, however higher vagal tone would be expected to have better behavioral outcomes, which is consistent with our findings.

The subcallosal cortex is involved with major depression (Hamani et al., 2011) and our findings revealed a deactivation following exercise, a similar response to anti-depressants (Mayberg et al., 2002) and suggests a potential mechanism for improving cognition indirectly by improving stress and mood (Bartholomew et al., 2005; Basso & Suzuki, 2017; Yeung, 1996). Interestingly, those with lower vagal tone experienced a greater magnitude of deactivation in

the subcallosal cortex following exercise, relative to rest, compared to those with higher vagal tone. Lower vagal tone is associated with poor emotional regulation (Porges, 2007; Smith et al., 2017) and this may be due to greater resting-state activity of the subcallosal cortex, thus having more room for improvement in response to exercise than those with higher vagal tone. Given that chronic exercise increases vagal tone (Albinet et al., 2016, 2010) and reduces depressive symptoms (Craft & Landers, 2016; Herring, 2012), perhaps the cumulative effect of each acute bout of exercise with improved moved and a reduction in activity in the subcallosal cortex may yield the chronic exercise downstream benefits.

Strengths and Limitations

This study has multiple strengths. This is the first study to date to investigate the mechanisms of BDNF and/or neural activation in the relationship of acute exercise and executive function in healthy older adults. The evidence provides a meaningful contribution to the extant literature by showing that exercise can affect BDNF, neural activation, and executive function in older adults and the change in executive function is related to change in BDNF and neural activation. However, without the inclusion of a meaningful individual factor (e.g. vagal tone), some of these findings would have been overshadowed by individual variability. To investigate these relationships, we utilized a within-subjects, counterbalanced design. There is strong individual variability in the responses to exercise for BDNF, neural activation, and executive function, as

demonstrated here, however using a within-subjects design allowed for powerful comparisons across conditions. To account for some of the individual variability, we proposed a novel moderator, vagal tone, in the relationship between acute exercise and executive function, BDNF, and neural activation; the use of this moderator proved to be important in separating the magnitude of benefits achieved with exercise.

Despite these strengths, this study is not without limitations. Our sample consisted of older adults who were of normal weight to overweight, cognitively normal, and did not have any major diseases or disorders. Further, our participants were highly active, with a mean of 592 minutes of MVPA per week, with only 3 out of the 16 participants not meeting the recommended guidelines for physical activity. The general physical activity guidelines for Americans recommend 150 minutes of MVPA per week. Although the percent of older adults who are meeting the recommended guidelines has increased in the past decade, only 42.5% of adults ages 65-74 years and 30.9% of adults ages 75-84 years are meeting the guidelines (CDC, 2014), which is substantially less than the sample included in this study.

Further, the median number of medications taken amongst adults 65 years and older is 4 (Charlesworth, Smit, Lee, Alramadhan, & Odden, 2015), whereas our participants took an average of 1.56 medications and 11 out of 16 took 0-1 prescribed medications. Therefore, our sample was unique as they were healthy, physically active older adults, which may limit the generalizability of our

findings. With that said, there was still heterogeneity within our sample for measures of resting vagal tone and response to exercise on measures of BDNF, neural activation, and executive function.

A further limitation is the use of a block design with fMRI. This was a limitation recognized prior to the start of the study but was a necessary delimitation because of the limited experience of the researcher with designing and/or utilizing event-related designs. A major disadvantage to the block design with cognitive testing is being predictable for participants with rapid habituation. With event-related designs, you have the ability to randomize each type of stimuli across a period of time. For example, with an event-related design, we could have tested the Stroop with randomized trial presentation such as: incongruent, incongruent, neutral, incongruent. The hemodynamic response would have been averaged separately for each trial type, only including correct responses, thus yielding activation by trial type.

For this study, the trial types were presented in separate blocks where all trials were of the same type within each block. Though this provides higher statistical power, we cannot throw out individual trials (i.e. error trials) and there is a chance that participants will habituate to the condition and find a successful strategy to perform the task. Even so, the block design was sensitive to condition-related changes and has been used in similar acute exercise and executive function with fMRI paradigms (Chen et al., 2016; Li et al., 2014).

Interestingly, the traditional version of the Stroop test that has been widely used in acute exercise studies is more similar to a block-design than to an event-related design. The traditional version can include the neutral, congruent, and incongruent stimuli types (as described in the methods), however instead of participants responding to as many single stimuli as they can over the course of 30 seconds, they have a list of stimuli that they respond to as quickly as possible. A major difference is in the traditional version, stimuli for each trial type are usually tested only once (e.g. participants respond to the entire list that they are given), whereas in the computer version, stimuli are tested for 30 seconds at a time and tested multiple times. Although the block design is more similar to the traditional version than event-related, there are still marked differences between the traditional and computerized versions and this limits the generalizability of these findings with similar studies using the traditional version.

The fMRI findings are further limited by the assumptions of the BOLD signal, which is dependent on neurovascular coupling. Neurovascular coupling links neuronal activity to increases in blood flow and is affected by factors that contribute to the constriction and dilation of the vasculature (Buxton, 2013). Age-related changes in neurovascular coupling are associated with increases in oxidative stress and endothelial dysfunction, which has downstream effects on the BOLD signal (Tarantini, Tran, Gordon, Ungvari, & Csiszar, 2017). Although the sample in this study were all older adults, there may have been individual differences in level of oxidative stress or other factors affecting the

responsiveness of the vasculature. Vagal tone is associated with oxidative stress in clinical patients (Fadaee et al., 2017; Pavithran, Nandeesha, Sathiyapriya, Bobby, & Madanmohan, 2008) and higher vagal tone is suggested to reflect a system that is resistant to oxidative stress (Gasparovic et al., 2017). The high and low vagal tone groups in this study may have differed in neural activation, as assessed by the BOLD signal, due to differences in neurovascular coupling rather than differences in neural activation per se. Future research should consider including heart rate variability, to be reduced to vagal tone, as the measurement is quick and cost efficient and may provide meaningful information about vascular dysfunction.

Lastly, the high and low vagal tone groups were not equated for sex which may have influenced the group differences. Although RMSSD is not considered to be a heart rate variability metric that is affected by sex (Koenig & Thayer, 2016), sex does have a substantial effect on other time and frequency domain metrics, and it is possible that the vagal tone groups were not independent of sex differences. Females have higher high frequency power than males (Koenig & Thayer, 2016) and high frequency power is highly correlated to RMSSD, consistent with the findings of this study. Therefore, although we may not expect RMSSD to be impacted by sex, the relation with high frequency power and RMSSD in addition to our high vagal tone group having more females than the low vagal one group may be suggestive of a sex influence. Future studies should consider equating groups for sex or separating males and females.

Conclusion

These findings present evidence of initial relationships involved in the acute exercise and executive function relationship in older adults. Exercise did not have a robust effect on executive function, however when including putative mediators (BDNF, neural activation) and a novel moderator (vagal tone) the effects of acute exercise became clearer. Although meta-analyses have shown benefits with acute exercise on executive function, this effect is of small magnitude and when considering potential moderators of this relationship (e.g. task type, exercise type) some effects become negligible (Chang et al., 2012; Lambourne & Tomporowski, 2010; Ludyga et al., 2016). Though some people benefit from exercise, there is great variability and understanding that variability is the first step into understanding how to target executive function in older adults.

Our findings suggest that the response to acute exercise differs between those with high and low vagal tone. Here if we had failed to include vagal tone, we would have concluded that acute exercise had no overall effect on executive function in this older adult population, however the benefits were dependent on changes in BDNF and neural activation; though accurate, that conclusion would have discounted important findings that aid in the understanding of this relationship. Moving beyond mean outcomes and exploring individual variability in response to acute exercise and in relation to chronic exercise adaptations is a necessary step to continue advancing our understanding of how exercise affects

cognition. Including individual factors that cannot be modified by exercise interventions (e.g. genetic factors, age, sex, higher education) and those that affect the acute response and can targeted by interventions (e.g. vagal tone) will help elucidate groups of people that respond to exercise at differing magnitudes, as well as identify individual factors that interventions should target to improve the outcomes.

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